EXHIBIT 344

Infectious diseases

Overview

Infectious diseases are disorders caused by organisms — such as bacteria, viruses, fungi or parasites. Many organisms live in and on our bodies. They're normally harmless or even helpful. But under certain conditions, some organisms may cause disease.

Some infectious diseases can be passed from person to person. Some are transmitted by insects or other animals. And you may get others by consuming contaminated food or water or being exposed to organisms in the environment.

Signs and symptoms vary depending on the organism causing the infection, but often include fever and fatigue. Mild infections may respond to rest and home remedies, while some life-threatening infections may need hospitalization.

Many infectious diseases, such as measles and chickenpox, can be prevented by vaccines. Frequent and thorough hand-washing also helps protect you from most infectious diseases.

Symptoms

Each infectious disease has its own specific signs and symptoms. General signs and symptoms common to a number of infectious diseases include:

- Fever
- Diarrhea
- Fatigue
- Muscle aches
- Coughing

When to see a doctor

Seek medical attention if you:

- Have been bitten by an animal
- Are having trouble breathing
- Have been coughing for more than a week
- Have severe headache with fever
- Experience a rash or swelling
- Have unexplained or prolonged fever
- Have sudden vision problems

Causes

Infectious diseases can be caused by:

- **Bacteria.** These one-cell organisms are responsible for illnesses such as strep throat, urinary tract infections and tuberculosis.
- **Viruses.** Even smaller than bacteria, viruses cause a multitude of diseases ranging from the common cold to AIDS.
- **Fungi.** Many skin diseases, such as ringworm and athlete's foot, are caused by fungi. Other types of fungi can infect your lungs or nervous system.
- **Parasites.** Malaria is caused by a tiny parasite that is transmitted by a mosquito bite. Other parasites may be transmitted to humans from animal feces.

Direct contact

An easy way to catch most infectious diseases is by coming in contact with a person or an animal with the infection. Infectious diseases can be spread through direct contact such as:

- **Person to person.** Infectious diseases commonly spread through the direct transfer of bacteria, viruses or other germs from one person to another. This can happen when an individual with the bacterium or virus touches, kisses, or coughs or sneezes on someone who isn't infected.

  These germs can also spread through the exchange of body fluids from sexual contact. The person who passes the germ may have no symptoms of the disease, but may simply be a carrier.

- **Animal to person.** Being bitten or scratched by an infected animal — even a pet — can make you sick and, in extreme circumstances, can be fatal. Handling animal waste can be

hazardous, too. For example, you can get a toxoplasmosis infection by scooping your cat's litter box.

- **Mother to unborn child.** A pregnant woman may pass germs that cause infectious diseases to her unborn baby. Some germs can pass through the placenta or through breast milk. Germs in the vagina can also be transmitted to the baby during birth.

Indirect contact

Disease-causing organisms also can be passed by indirect contact. Many germs can linger on an inanimate object, such as a tabletop, doorknob or faucet handle.

When you touch a doorknob handled by someone ill with the flu or a cold, for example, you can pick up the germs he or she left behind. If you then touch your eyes, mouth or nose before washing your hands, you may become infected.

Insect bites

Some germs rely on insect carriers — such as mosquitoes, fleas, lice or ticks — to move from host to host. These carriers are known as vectors. Mosquitoes can carry the malaria parasite or West Nile virus. Deer ticks may carry the bacterium that causes Lyme disease.

Food contamination

Disease-causing germs can also infect you through contaminated food and water. This mechanism of transmission allows germs to be spread to many people through a single source. Escherichia coli (E. coli), for example, is a bacterium present in or on certain foods — such as undercooked hamburger or unpasteurized fruit juice.

Risk factors

While anyone can catch infectious diseases, you may be more likely to get sick if your immune system isn't working properly. This may occur if:

- You're taking steroids or other medications that suppress your immune system, such as anti-rejection drugs for a transplanted organ
- You have HIV or AIDS
- You have certain types of cancer or other disorders that affect your immune system

In addition, certain other medical conditions may predispose you to infection, including implanted medical devices, malnutrition and extremes of age, among others.

Complications

Most infectious diseases have only minor complications. But some infections — such as pneumonia, AIDS and meningitis — can become life-threatening. A few types of infections have been linked to a long-term increased risk of cancer:

- Human papillomavirus is linked to cervical cancer
- Helicobacter pylori is linked to stomach cancer and peptic ulcers
- Hepatitis B and C have been linked to liver cancer

In addition, some infectious diseases may become silent, only to appear again in the future — sometimes even decades later. For example, someone who's had chickenpox may develop shingles much later in life.

Prevention

Follow these tips to decrease the risk of infection:

- **Wash your hands.** This is especially important before and after preparing food, before eating, and after using the toilet. And try not to touch your eyes, nose or mouth with your hands, as that's a common way germs enter the body.
- **Get vaccinated.** Vaccination can drastically reduce your chances of contracting many diseases. Make sure to keep up to date on your recommended vaccinations, as well as your children's.
- **Stay home when ill.** Don't go to work if you are vomiting, have diarrhea or have a fever. Don't send your child to school if he or she has these signs, either.
- **Prepare food safely.** Keep counters and other kitchen surfaces clean when preparing meals. Cook foods to the proper temperature, using a food thermometer to check for doneness. For ground meats, that means at least 160 F (71 C); for poultry, 165 F (74 C); and for most other meats, at least 145 F (63 C).

  Also promptly refrigerate leftovers — don't let cooked foods remain at room temperature for long periods of time.
- **Practice safe sex.** Always use condoms if you or your partner has a history of sexually transmitted infections or high-risk behavior.
- **Don't share personal items.** Use your own toothbrush, comb and razor. Avoid sharing drinking glasses or dining utensils.
- **Travel wisely.** If you're traveling out of the country, talk to your doctor about any special

vaccinations — such as yellow fever, cholera, hepatitis A or B, or typhoid fever — you may need.

By Mayo Clinic Staff

EXHIBIT 345

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Immunization Schedules

Table 1. Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2020

Always make recommendations by determining needed vaccines based on age (Table 1), determining appropriate intervals for catch-up, if needed (Table 2), assessing for medical indications (Table 3), and reviewing special situations (Notes).

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Table 1. By age | Table 2. Catch-up schedule | Table 3. By medical indications | Schedule Changes & Guidance | Parent-friendly schedule | Resources for health care providers

- 8.5″x11″ print color [8 pages]
- 8.5″x11″ print black and white [8 pages]
- Compliant version of this schedule
- Vaccines in the Child and Adolescent Immunization Schedule
- Learn how to display current schedules from your website.

Download Schedules App

Legend

Range of recommended ages for all children	Range of recommended ages for catch-up immunization	Range of recommended ages for certain high-risk groups	Recommended based on shared clinical decision-making or *can be used in this age group	No recommendation/Not applicable

Birth to 15 Months

Vaccine	Birth	1 mo	2 mos	4 mos	6 mos	9 mos	12 mos	15 mos
Hepatitis B (HepB)	1st dose	2nd dose			←3rd dose→			

Rotavirus (RV) RV1 (2-dose series); RV5 (3-dose series)			1st dose	2nd dose	See notes			
Diphtheria, tetanus, & acellular pertussis (DTaP: <7 yrs)			1st dose	2nd dose	3rd dose			←4th dose→
***Haemophilus influenzae* type b** (Hib)			1st dose	2nd dose	See notes		←3rd or 4th dose, See notes→	
Pneumococcal conjugate (PCV13)			1st dose	2nd dose	3rd dose		←4th dose→	
Inactivated poliovirus (IPV: <18 yrs)			1st dose	2nd dose	←3rd dose→			
Influenza (IIV)					Annual vaccination 1 or 2 doses			
or **Influenza** (LAIV)								
Measles, mumps, rubella (MMR)					See notes		←1st dose→	
Varicella (VAR)							←1st dose→	
Hepatitis A (HepA)					See notes		←2-dose series, See notes→	
Tetanus, diphtheria, & acellular pertussis (Tdap: ≥7 yrs)								
Human papillomavirus (HPV)								
Meningococcal (MenACWY-D: ≥9 mos; MenACWY-CRM: ≥2 mos)			See notes					
Meningococcal B (MenB)								
Pneumococcal polysaccharide (PPSV23)								

18 Months to 18 Years

Vaccines	18 mos	19-23 mos	2-3 yrs	4-6 yrs	7-10 yrs	11-12 yrs	13-15 yrs	16 yrs	17-18 yrs
Hepatitis B (HepB)	←3rd dose→								
Rotavirus (RV) RV1 (2-dose series); RV5 (3-dose series)									
Diphtheria, tetanus, & acellular pertussis (DTaP: <7 yrs)	←4th dose→			5th dose					
***Haemophilus influenzae* type b** (Hib)									
Pneumococcal conjugate (PCV13)									
Inactivated poliovirus (IPV: <18 yrs)	←3rd dose→			4th dose					
Influenza (IIV)	Annual vaccination 1 or 2 doses					Annual vaccination 1 dose only			
or **Influenza** (LAIV)			Annual vaccination 1 or 2 doses		or	Annual vaccination 1 dose only			
Measles, mumps, rubella (MMR)				2nd dose					
Varicella (VAR)				2nd dose					
Hepatitis A (HepA)	← 2-dose series, See notes→								
Tetanus, diphtheria, &						Tdap			

acellular pertussis (Tdap: ≥7 yrs)								
Human papillomavirus (HPV)						*	See notes	
Meningococcal (MenACWY-D: ≥9 mos; MenACWY-CRM: ≥2 mos)	See notes						1st dose	2nd dose
Meningococcal B (MenB)						See notes		
Pneumococcal polysaccharide (PPSV23)			See notes					

Administer recommended vaccines if immunization history is incomplete or unknown. Do not restart or add doses to vaccine series for extended intervals between doses. When a vaccine is not administered at the recommended age, administer at a subsequent visit. The use of trade names is for identification purposes only and does not imply endorsement by the ACIP or CDC.

Notes

Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2020

For vaccine recommendations for persons 19 years of age or older, see the Recommended Adult Immunization Schedule.

Additional information

- Consult relevant ACIP statements for detailed recommendations.
- For information on contraindications and precautions for the use of a vaccine, consult the General Best Practice Guidelines for Immunization and relevant ACIP statements.
- For calculating intervals between doses, 4 weeks = 28 days. Intervals of ≥4 months are determined by calendar months.
- Within a number range (e.g., 12–18), a dash (–) should be read as "through."
- Vaccine doses administered ≤4 days before the minimum age or interval are considered valid. Doses of any vaccine administered ≥5 days earlier than the minimum age or minimum interval should not be counted as valid and should be repeated as age-appropriate. The repeat dose should be spaced after the invalid dose by the recommended minimum interval. For further details, see Table 3-1, Recommended and minimum ages and intervals between

vaccine doses, in General Best Practice Guidelines for Immunization.

- Information on travel vaccine requirements and recommendations is available at wwwnc.cdc.gov/travel/.
- For vaccination of persons with immunodeficiencies, see Table 8-1, Vaccination of persons with primary and secondary immunodeficiencies, in General Best Practice Guidelines for Immunization, and Immunization in Special Clinical Circumstances (In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. *Red Book: 2018 report of the Committee on Infectious Diseases*. 31st ed. Itasca, IL: American Academy of Pediatrics, 2018:67–111).
- For information regarding vaccination in the setting of a vaccine-preventable disease outbreak, contact your state or local health department.
- The National Vaccine Injury Compensation Program (VICP) is a no-fault alternative to the traditional legal system for resolving vaccine injury claims. All routine child and adolescent vaccines are covered by VICP except for pneumococcal polysaccharide vaccine (PPSV23). For more information, see www.hrsa.gov/vaccinecompensation/index.html .

Diphtheria, tetanus, and pertussis (DTaP) vaccination (minimum age: 6 weeks [4 years for Kinrix or Quadracel])

Routine vaccination

- 5-dose series at 2, 4, 6, 15–18 months, 4–6 years
  - **Prospectively:** Dose 4 may be administered as early as age 12 months if at least 6 months have elapsed since dose 3.
  - **Retrospectively:** A 4th dose that was inadvertently administered as early as 12 months may be counted if at least 4 months have elapsed since dose 3.

Catch-up vaccination

- Dose 5 is not necessary if dose 4 was administered at age 4 years or older and at least 6 months after dose 3.
- For other catch-up guidance, see Table 2.

Haemophilus influenzae type b vaccination (minimum age: 6 weeks)

Routine vaccination

- **ActHIB, Hiberix, or Pentacel:** 4-dose series at 2, 4, 6, 12–15 months
- **PedvaxHIB:** 3-dose series at 2, 4, 12–15 months

Catch-up vaccination

- **Dose 1 at 7–11 months:** Administer dose 2 at least 4 weeks later and dose 3 (final dose) at 12–15 months or 8 weeks after dose 2 (whichever is later).
- **Dose 1 at 12–14 months:** Administer dose 2 (final dose) at least 8 weeks after dose 1.

- **Dose 1 before 12 months and dose 2 before 15 months:** Administer dose 3 (final dose) 8 weeks after dose 2.
- **2 doses of PedvaxHIB before 12 months:** Administer dose 3 (final dose) at 12–59 months and at least 8 weeks after dose 2.
- **Unvaccinated at 15–59 months:** 1 dose
- **Previously unvaccinated children age 60 months or older** who are not considered high risk do not require catch-up vaccination.
- For other catch-up guidance, see Table 2.

Special situations

- **Chemotherapy or radiation treatment:**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Doses administered within 14 days of starting therapy or during therapy should be repeated at least 3 months after therapy completion.
- **Hematopoietic stem cell transplant (HSCT):**
  - 3-dose series 4 weeks apart starting 6 to 12 months after successful transplant regardless of Hib vaccination history
- **Anatomic or functional asplenia (including sickle cell disease):**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Unvaccinated* persons age 5 years or older
  - 1 dose
- **Elective splenectomy:**
  Unvaccinated* persons age 15 months or older
  - 1 dose (preferably at least 14 days before procedure)
- **HIV infection:**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Unvaccinated* persons age 5–18 years
  - 1 dose
- **Immunoglobulin deficiency, early component complement deficiency:**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

*Unvaccinated = Less than routine series (through 14 months) OR no doses (15 months or older)

Hepatitis A vaccination (minimum age: 12 months for routine vaccination)

Routine vaccination

- 2-dose series (minimum interval: 6 months) beginning at age 12 months

Catch-up vaccination

- Unvaccinated persons through 18 years should complete a 2-dose series (minimum interval: 6 months).
- Persons who previously received 1 dose at age 12 months or older should receive dose 2 at least 6 months after dose 1.
- Adolescents 18 years and older may receive the combined HepA and HepB vaccine, **Twinrix®**, as a 3-dose series (0, 1, and 6 months) or 4-dose series (0, 7, and 21–30 days, followed by a dose at 12 months).

International travel

- Persons traveling to or working in countries with high or intermediate endemic hepatitis A:
  - **Infants age 6–11 months:** 1 dose before departure; revaccinate with 2 doses, separated by at least 6 months, between 12 and 23 months of age
  - **Unvaccinated age 12 months and older:** Administer dose 1 as soon as travel is considered.

Hepatitis B vaccination (minimum age: birth)

Birth dose (monovalent HepB vaccine only)

- **Mother is HBsAg-negative:** 1 dose within 24 hours of birth for **all** medically stable infants ≥2,000 grams. Infants <2,000 grams: administer 1 dose at chronological age 1 month or hospital discharge.
- **Mother is HBsAg-positive:**
  - Administer **HepB vaccine** and **hepatitis B immune globulin (HBIG)** (in separate limbs) within 12 hours of birth, regardless of birth weight. For infants <2,000 grams, administer 3 additional doses of vaccine (total of 4 doses) beginning at age 1 month.
  - Test for HBsAg and anti-HBs at age 9–12 months. If HepB series is delayed, test 1–2 months after final dose.
- **Mother's HBsAg status is unknown:**
  - Administer **HepB vaccine** within 12 hours of birth, regardless of birth weight.
  - For infants <2,000 grams, administer **HBIG** in addition to HepB vaccine (in separate limbs) within 12 hours of birth. Administer 3 additional doses of vaccine (total of 4 doses) beginning at age 1 month.
  - Determine mother's HBsAg status as soon as possible. If mother is HBsAg-positive, administer **HBIG** to infants ≥2,000 grams as soon as possible, but no later than 7 days of age.

Routine series

- 3-dose series at 0, 1–2, 6–18 months (use monovalent HepB vaccine for doses administered before age 6 weeks)
- Infants who did not receive a birth dose should begin the series as soon as feasible (see Table 2).
- Administration of **4 doses** is permitted when a combination vaccine containing HepB is used after the birth dose.
- **Minimum age** for the final (3rd or 4th) dose: 24 weeks
- **Minimum intervals:** dose 1 to dose 2: 4 weeks / dose 2 to dose 3: 8 weeks / dose 1 to dose 3: 16 weeks (when 4 doses are administered, substitute "dose 4" for "dose 3" in these calculations)

Catch-up vaccination

- Unvaccinated persons should complete a 3-dose series at 0, 1–2, 6 months.
- Adolescents age 11–15 years may use an alternative 2-dose schedule with at least 4 months between doses (adult formulation **Recombivax HB** only).
- Adolescents 18 years and older may receive a 2-dose series of HepB (**Heplisav-B®**) at least 4 weeks apart.
- Adolescents 18 years and older may receive the combined HepA and HepB vaccine, **Twinrix**, as a 3-dose series (0, 1, and 6 months) or 4-dose series (0, 7, and 21–30 days, followed by a dose at 12 months).
- For other catch-up guidance, see Table 2.

Special situations

- Revaccination is not generally recommended for persons with a normal immune status who were vaccinated as infants, children, adolescents, or adults.
- **Revaccination** may be recommended for certain populations, including:
  - **Infants born to HBsAg-positive mothers**
  - **Hemodialysis patients**
  - **Other immunocompromised persons**
- For detailed revaccination recommendations, please see the HepB *MMWR* publications.

Human papillomavirus vaccination (minimum age: 9 years)

Routine and catch-up vaccination

- HPV vaccination routinely recommended at **age 11–12 years (can start at age 9 years)** and catch-up HPV vaccination recommended for all persons through age 18 years if not adequately vaccinated
- 2- or 3-dose series depending on age at initial vaccination:
  - **Age 9 through 14 years at initial vaccination:** 2-dose series at 0, 6–12 months (minimum interval: 5 months; repeat dose if administered too soon)
  - **Age 15 years or older at initial vaccination:** 3-dose series at 0, 1–2 months, 6 months (minimum intervals: dose 1 to dose 2: 4 weeks / dose 2 to dose 3: 12 weeks / dose 1 to dose 3: 5 months; repeat dose if administered too soon)
- If completed valid vaccination series with any HPV vaccine, no additional doses needed

Special situations

- **Immunocompromising conditions, including HIV infection:** 3-dose series as above
- **History of sexual abuse or assault:** Start at age 9 years
- **Pregnancy:** HPV vaccination not recommended until after pregnancy; no intervention needed if vaccinated while pregnant; pregnancy testing not needed before vaccination

Influenza vaccination (minimum age: 6 months [IIV], 2 years [LAIV], 18 years [recombinant influenza vaccine, RIV])

Routine vaccination

- Use any influenza vaccine appropriate for age and health status annually:
  - 2 doses, separated by at least 4 weeks, for **children age 6 months–8 years** who have received fewer than 2 influenza vaccine doses before July 1, 2019, or whose influenza vaccination history is unknown (administer dose 2 even if the child turns 9 between receipt of dose 1 and dose 2)
  - 1 dose for **children age 6 months–8 years** who have received at least 2 influenza vaccine doses before July 1, 2019
  - 1 dose for **all persons age 9 years and older**
- For the 2020–21 season, see the 2020–21 ACIP influenza vaccine recommendations.

Special situations

- **Egg allergy, hives only**: Any influenza vaccine appropriate for age and health status annually
- **Egg allergy with symptoms other than hives** (e.g., angioedema, respiratory distress, need for emergency medical services or epinephrine): Any influenza vaccine appropriate for age and health status annually in medical setting under supervision of health care provider who can recognize and manage severe allergic reactions
- **LAIV should not be used** in persons with the following conditions or situations:
  - History of severe allergic reaction to a previous dose of any influenza vaccine or to any vaccine component (excluding egg, see details above)
  - Receiving aspirin or salicylate-containing medications
  - Age 2–4 years with history of asthma or wheezing
  - Immunocompromised due to any cause (including medications and HIV infection)
  - Anatomic or functional asplenia
  - Cochlear implant
  - Cerebrospinal fluid-oropharyngeal communication
  - Close contacts or caregivers of severely immunosuppressed persons who require a protected environment
  - Pregnancy
  - Received influenza antiviral medications within the previous 48 hours

Measles, mumps, and rubella vaccination (minimum age: 12 months for routine vaccination)

Routine vaccination

- 2-dose series at 12–15 months, 4–6 years
- Dose 2 may be administered as early as 4 weeks after dose 1.

Catch-up vaccination

- Unvaccinated children and adolescents: 2-dose series at least 4 weeks apart
- The maximum age for use of MMRV is 12 years.

Special situations

International travel

- **Infants age 6–11 months:** 1 dose before departure; revaccinate with 2-dose series with dose 1 at 12–15 months (12 months for children in high-risk areas) and dose 2 as early as 4 weeks later.
- **Unvaccinated children age 12 months and older:** 2-dose series at least 4 weeks apart before departure

Meningococcal serogroup A,C,W,Y vaccination (minimum age: 2 months [MenACWY-CRM, Menveo], 9 months [MenACWY-D, Menactra])

Routine vaccination

- 2-dose series at 11–12 years, 16 years

Catch-up vaccination

- Age 13–15 years: 1 dose now and booster at age 16–18 years (minimum interval: 8 weeks)
- Age 16–18 years: 1 dose

Special situations

Anatomic or functional asplenia (including sickle cell disease), HIV infection, persistent complement component deficiency, complement inhibitor (e.g., eculizumab, ravulizumab) use:

- **Menveo**
  - Dose 1 at age 8 weeks: 4-dose series at 2, 4, 6, 12 months
  - Dose 1 at age 7–23 months: 2-dose series (dose 2 at least 12 weeks after dose 1 and after age 12 months)

- Dose 1 at age 24 months or older: 2-dose series at least 8 weeks apart

- **Menactra**
  - **Persistent complement component deficiency or complement inhibitor use:**
    - Age 9–23 months: 2-dose series at least 12 weeks apart
    - Age 24 months or older: 2-dose series at least 8 weeks apart
  - **Anatomic or functional asplenia, sickle cell disease, or HIV infection:**
    - Age 9–23 months: Not recommended
    - Age 24 months or older: 2-dose series at least 8 weeks apart
    - **Menactra** must be administered at least 4 weeks after completion of PCV13 series.

Travel in countries with hyperendemic or epidemic meningococcal disease, including countries in the African meningitis belt or during the Hajj:

- Children less than age 24 months:
  - **Menveo (age 2–23 months):**
    - Dose 1 at 8 weeks: 4-dose series at 2, 4, 6, 12 months
    - Dose 1 at 7–23 months: 2-dose series (dose 2 at least 12 weeks after dose 1 and after age 12 months)
  - **Menactra (age 9–23 months):**
    - 2-dose series (dose 2 at least 12 weeks after dose 1; dose 2 may be administered as early as 8 weeks after dose 1 in travelers)
- Children age 2 years or older: 1 dose **Menveo** or **Menactra**

First-year college students who live in residential housing (if not previously vaccinated at age 16 years or older) or military recruits:

- 1 dose **Menveo** or **Menactra**

Adolescent vaccination of children who received MenACWY prior to age 10 years:

- **Children for whom boosters are recommended** because of an ongoing increased risk of meningococcal disease (e.g., those with complement deficiency, HIV, or asplenia): Follow the booster schedule for persons at increased risk (see below).
- **Children for whom boosters are not recommended** (e.g., those who received a single dose for travel to a country where meningococcal disease is endemic): Administer MenACWY according to the recommended adolescent schedule with dose 1 at age 11–12 years and dose 2 at age 16 years.

Note: Menactra should be administered either before or at the same time as DTaP. For MenACWY **booster dose recommendations** for groups listed under "Special situations" and in an outbreak setting and for additional meningococcal vaccination information, see meningococcal *MMWR* publications.

Meningococcal serogroup B vaccination (minimum age: 10 years [MenB-4C, Bexsero; MenB-FHbp, Trumenba])

Shared Clinical Decision-Making

- **Adolescents not at increased risk** age 16–23 years (preferred age 16–18 years) based on shared clinical decision-making:
  - **Bexsero:** 2-dose series at least 1 month apart
  - **Trumenba:** 2-dose series at least 6 months apart; if dose 2 is administered earlier than 6 months, administer a 3rd dose at least 4 months after dose 2.

Special situations

Anatomic or functional asplenia (including sickle cell disease), persistent complement component deficiency, complement inhibitor (e.g., eculizumab, ravulizumab) use:

- **Bexsero:** 2-dose series at least 1 month apart
- **Trumenba:** 3-dose series at 0, 1–2, 6 months

Bexsero and **Trumenba** are not interchangeable; the same product should be used for all doses in a series. For MenB **booster dose recommendations** for groups listed under "Special situations" and in an outbreak setting and for additional meningococcal vaccination information, see ACIP Recommendations.

Pneumococcal vaccination (minimum age: 6 weeks [PCV13], 2 years [PPSV23])

Routine vaccination with PCV13

- 4-dose series at 2, 4, 6, 12–15 months

Catch-up vaccination with PCV13

- 1 dose for healthy children age 24–59 months with any incomplete* PCV13 series
- For other catch-up guidance, see Table 2.

Special situations

High-risk conditions below: When both PCV13 and PPSV23 are indicated, administer PCV13 first. PCV13 and PPSV23 should not be administered during the same visit.

Chronic heart disease (particularly cyanotic congenital heart disease and cardiac failure), chronic lung disease (including asthma treated with high-dose, oral corticosteroids), diabetes mellitus:

Age 2–5 years

- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)

- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Age 6–18 years

- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Cerebrospinal fluid leak, cochlear implant:

Age 2–5 years

- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Age 6–18 years

- No history of either PCV13 or PPSV23: 1 dose PCV13, 1 dose PPSV23 at least 8 weeks later
- Any PCV13 but no PPSV23: 1 dose PPSV23 at least 8 weeks after the most recent dose of PCV13
- PPSV23 but no PCV13: 1 dose PCV13 at least 8 weeks after the most recent dose of PPSV23

Sickle cell disease and other hemoglobinopathies; anatomic or functional asplenia; congenital or acquired immunodeficiency; HIV infection; chronic renal failure; nephrotic syndrome; malignant neoplasms, leukemias, lymphomas, Hodgkin disease, and other diseases associated with treatment with immunosuppressive drugs or radiation therapy; solid organ transplantation; multiple myeloma:

Age 2–5 years

- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose) and a 2nd dose of PPSV23 5 years later

Age 6–18 years

- No history of either PCV13 or PPSV23: 1 dose PCV13, 2 doses PPSV23 (dose 1 of PPSV23 administered 8 weeks after PCV13 and dose 2 of PPSV23 administered at least 5 years after dose 1 of PPSV23)
- Any PCV13 but no PPSV23: 2 doses PPSV23 (dose 1 of PPSV23 administered 8 weeks after the most recent dose of PCV13 and dose 2 of PPSV23 administered at least 5 years after dose 1 of PPSV23)
- PPSV23 but no PCV13: 1 dose PCV13 at least 8 weeks after the most recent PPSV23 dose and a 2nd dose of PPSV23 administered 5 years after dose 1 of PPSV23 and at least 8 weeks after a dose of PCV13

Chronic liver disease, alcoholism:

Age 6–18 years

- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

*Incomplete series = Not having received all doses in either the recommended series or an age-appropriate catch-up series. See Tables 8, 9, and 11 in the ACIP pneumococcal vaccine recommendations [24 pages] for complete schedule details.

Poliovirus vaccination (minimum age: 6 weeks)

Routine vaccination

- 4-dose series at ages 2, 4, 6–18 months, 4–6 years; administer the final dose at or after age 4 years and at least 6 months after the previous dose.
- 4 or more doses of IPV can be administered before age 4 years when a combination vaccine containing IPV is used. However, a dose is still recommended at or after age 4 years and at least 6 months after the previous dose.

Catch-up vaccination

- In the first 6 months of life, use minimum ages and intervals only for travel to a polio-endemic region or during an outbreak.
- IPV is not routinely recommended for U.S. residents 18 years and older.

Series containing oral polio vaccine (OPV), either mixed OPV-IPV or OPV-only series:

- Total number of doses needed to complete the series is the same as that recommended for the U.S. IPV schedule. See Guidance for Assessment of Poliovirus Vaccination Status and Vaccination of Children Who Have Received Poliovirus Vaccine Outside the United States.
- Only trivalent OPV (tOPV) counts toward the U.S. vaccination requirements.
  - Doses of OPV administered before April 1, 2016, should be counted (unless specifically noted as administered during a campaign).
  - Doses of OPV administered on or after April 1, 2016, should not be counted.
  - For guidance to assess doses documented as "OPV," see Errata: Vol. 66, No. 1.
- For other catch-up guidance, see Table 2.

Rotavirus vaccination (minimum age: 6 weeks)

Routine vaccination

- **Rotarix:** 2-dose series at 2 and 4 months
- **RotaTeq:** 3-dose series at 2, 4, and 6 months
- If any dose in the series is either **RotaTeq** or unknown, default to 3-dose series.

Catch-up vaccination

- Do not start the series on or after age 15 weeks, 0 days.
- The maximum age for the final dose is 8 months, 0 days.

- For other catch-up guidance, see Table 2.

Tetanus, diphtheria, and pertussis (Tdap) vaccination (minimum age: 11 years for routine vaccination, 7 years for catch-up vaccination)

Routine vaccination

- **Adolescents age 11–12 years:** 1 dose Tdap
- **Pregnancy:** 1 dose Tdap during each pregnancy, preferably in early part of gestational weeks 27–36
- Tdap may be administered regardless of the interval since the last tetanus- and diphtheria-toxoid-containing vaccine.

Catch-up vaccination

- **Adolescents age 13–18 years who have not received Tdap:** 1 dose Tdap, then Td or Tdap booster every 10 years
- **Persons age 7–18 years not fully vaccinated* with DTaP:** 1 dose Tdap as part of the catch-up series (preferably the first dose); if additional doses are needed, use Td or Tdap.
- **Tdap administered at 7–10 years**
  - **Children age 7–9 years** who receive Tdap should receive the routine Tdap dose at age 11–12 years.
  - **Children age 10 years** who receive Tdap do not need to receive the routine Tdap dose at age 11–12 years.
- **DTaP inadvertently administered at or after age 7 years:**
  - **Children age 7–9 years:** DTaP may count as part of catch-up series. Routine Tdap dose at age 11–12 years should be administered.
  - **Children age 10–18 years:** Count dose of DTaP as the adolescent Tdap booster.
- For other catch-up guidance, see Table 2.
- For information on use of Tdap or Td as tetanus prophylaxis in wound management, see Prevention of Pertussis, Tetanus, and Diphtheria with Vaccines in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP).

*Fully vaccinated = 5 valid doses of DTaP OR 4 valid doses of DTaP if dose 4 was administered at age 4 years or older.

Varicella vaccination (minimum age: 12 months)

Routine vaccination

- 2-dose series at 12–15 months, 4–6 years
- Dose 2 may be administered as early as 3 months after dose 1 (a dose administered after a 4-week interval may be counted).

Catch-up vaccination

- Ensure persons age 7–18 years without evidence of immunity (see *MMWR* [48 pages]) have 2-dose series:
  - **Age 7–12 years:** routine interval: 3 months (a dose administered after a 4-week interval may be counted)
  - **Age 13 years and older:** routine interval: 4–8 weeks (minimum interval: 4 weeks)
  - The maximum age for use of MMRV is 12 years.

Vaccines in the Child and Adolescent Immunization Schedule

Vaccines	Abbreviations	Trade Names
Diphtheria, tetanus, and acellular pertussis vaccine	DTaP	Daptacel® Infanrix®
Diphtheria, tetanus vaccine	DT	No Trade Name
***Haemophilus influenzae* type B vaccine**	Hib (PRP-T) Hib (PRP-OMP)	ActHIB® Hiberix® PedvaxHIB®
Hepatitis A vaccine	HepA	Havrix® Vaqta®
Hepatitis B vaccine	HepB	Engerix-B® Recombivax HB®
Human papillomavirus vaccine	HPV	Gardasil 9®
Influenza vaccine (inactivated)	IIV	Multiple
Influenza vaccine (live, attenuated)	LAIV	FluMist® Quadrivalent
Measles, mumps, and rubella vaccine	MMR	M-M-R® II
Meningococcal serogroups A, C, W, Y vaccine	MenACWY-D MenACWY-CRM	Menactra® Menveo®
Meningococcal serogroup B vaccine	MenB-4C MenB-FHbp	Bexsero® Trumenba®
Pneumococcal 13-valent conjugate vaccine	PCV13	Prevnar 13®
Pneumococcal 23-valent polysaccharide vaccine	PPSV23	Pneumovax® 23

Poliovirus vaccine (inactivated)	IPV	IPOL®
Rotavirus vaccine	RV1 RV5	Rotarix® RotaTeq®
Tetanus, diphtheria, and acellular pertussis vaccine	Tdap	Adacel® Boostrix®
Tetanus and diphtheria vaccine	Td	Tenivac® TDvax™
Varicella vaccine	VAR	Varivax®

Combination Vaccines

(Use combination vaccines instead of separate injections when appropriate)

Vaccines	Abbreviations	Trade Names
DTaP, hepatitis B, and inactivated poliovirus vaccine	DTaP-HepB-IPV	Pediarix®
DTaP, inactivated poliovirus, and *Haemophilus influenzae* type B vaccine	DTaP-IPV/Hib	Pentacel®
DTaP and inactivated poliovirus vaccine	DTaP-IPV	Kinrix® Quadracel®
Measles, mumps, rubella, and varicella vaccines	MMRV	ProQuad®

This schedule is recommended by the Advisory Committee on Immunization Practices (ACIP) and approved by the Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), American Academy of Family Physicians (AAFP), American College of Obstetricians and Gynecologists (ACOG), and American College of Nurse-Midwives (ACNM).

The comprehensive summary of the ACIP recommended changes made to the child and adolescent immunization schedule can be found in the February 6, 2020 *MMWR*.

Report

- Suspected cases of reportable vaccine-preventable diseases or outbreaks to your state or local health department
- Clinically significant adverse events to the Vaccine Adverse Event Reporting System (VAERS) at www.vaers.hhs.gov or (800-822-7967)

Helpful information

- Complete ACIP recommendations

- General Best Practice Guidelines for Immunization
- Outbreak information (including case identification and outbreak response), see Manual for the Surveillance of Vaccine-Preventable Diseases

Page last reviewed: February 3, 2020
Content source: National Center for Immunization and Respiratory Diseases

EXHIBIT 346

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Immunization Schedules

Table 1. Recommended Adult Immunization Schedule for ages 19 years or older, United States, 2020

Always make recommendations by determining needed vaccines based on age (Table 1), assessing for medical conditions and other indications (Table 2), and reviewing special situations (Notes).

Get Email Updates

Table 1. By age | Table 2. By indications | Schedule Changes & Guidance | Resources for health care | Resources for adults | Download schedules app

- 8.5″x11″ print color [6 pages]
- 8.5″x11″ print black and white [6 pages]
- Compliant version of this schedule
- Vaccines in the Adult Immunization Schedule
- Learn how to display current schedules from your website.
- Hard copies of the schedule are available for free using the CDC-info on Demand order form.

Download Schedules App

Legend

Recommended vaccination for adults who meet age requirement, lack documentation of vaccination, or lack evidence of past infection	Recommended vaccination for adults with an additional risk factor or another indication	Recommended vaccination based on shared clinical decision-making

No recommendation/Not applicable

Vaccine	19-26 years	27-49 years	50-64 years	≥65 years
Influenza inactivated (IIV) or	1 dose annually			

Influenza recombinant (RIV)				
or **Influenza live attenuated (LAIV)**		or 1 dose annually		
Tetanus, diphtheria, pertussis (Tdap or Td)	1 dose Tdap, then Td or Tdap booster every 10 yrs			
Measles, mumps, rubella (MMR)	1 or 2 doses depending on indication (if born in 1957 or later)			
Varicella (VAR)	2 doses (if born in 1980 or later)		2 doses	
Zoster recombinant (RZV) (preferred)				2 doses
or **Zoster live (ZVL)**				or 1 dose
Human papillomavirus (HPV)	2 or 3 doses depending on age at initial vaccination or condition	27 through 45 years		
Pneumococcal conjugate (PCV13)	1 dose			65 years and older
Pneumococcal polysaccharide (PPSV23)	1 or 2 doses depending on indication			1 dose
Hepatitis A (HepA)	2 or 3 doses depending on vaccine			
Hepatitis B	2 or 3 doses depending on vaccine			

(HepB)		
Meningococcal A, C, W, Y (MenACWY)	1 or 2 doses depending on indication, see notes for booster recommendations	
Meningococcal B (MenB)		2 or 3 doses depending on vaccine and indication, see notes for booster recommendations
	19 through 23 years	
***Haemophilus influenzae* type b (Hib)**	1 or 3 doses depending on indication	

Administer recommended vaccines if vaccination history is incomplete or unknown. Do not restart or add doses to vaccine series if there are extended intervals between doses. The use of trade names is for identification purposes only and does not imply endorsement by the ACIP or CDC.

Notes

Recommended Adult Immunization Schedule for ages 19 years or older, United States, 2020

For vaccine recommendations for persons age 0 through 18 years, see the Child and Adolescent Immunization Schedule.

Haemophilus influenzae type b vaccination

Special situations

- **Anatomical or functional asplenia (including sickle cell disease)**: 1 dose if previously did not receive Hib; if elective splenectomy, 1 dose, preferably at least 14 days before splenectomy
- **Hematopoietic stem cell transplant** (HSCT): 3-dose series 4 weeks apart starting 6–12 months after successful transplant, regardless of Hib vaccination history

Hepatitis A vaccination

Routine vaccination

- **Not at risk but want protection from hepatitis A** (identification of risk factor not required): 2-dose series HepA

(Havrix 6–12 months apart or Vaqta 6–18 months apart [minimum interval: 6 months]) or 3-dose series HepA-HepB (Twinrix at 0, 1, 6 months [minimum intervals: 4 weeks between doses 1 and 2, 5 months between doses 2 and 3])

Special situations

- **At risk for hepatitis A virus infection**: 2-dose series HepA or 3-dose series HepA-HepB as above
  - **Chronic liver disease** (e.g., persons with hepatitis B, hepatitis C, cirrhosis, fatty liver disease, alcoholic liver disease, autoimmune hepatitis, alanine aminotransferase [ALT] or aspartate aminotransferase [AST] level greater than twice the upper limit of normal)
  - **HIV infection**
  - **Men who have sex with men**
  - **Injection or noninjection drug use**
  - **Persons experiencing homelessness**
  - **Work with hepatitis A virus** in research laboratory or with nonhuman primates with hepatitis A virus infection
  - **Travel in countries with high or intermediate endemic hepatitis A**
  - **Close, personal contact with international adoptee** (e.g., household or regular babysitting) in first 60 days after arrival from country with high or intermediate endemic hepatitis A (administer dose 1 as soon as adoption is planned, at least 2 weeks before adoptee's arrival)
  - **Pregnancy** if at risk for infection or severe outcome from infection during pregnancy
  - **Settings for exposure, including** health care settings targeting services to injection or noninjection drug users or group homes and nonresidential day care facilities for developmentally disabled persons (individual risk factor screening not required)

Hepatitis B vaccination

Routine vaccination

- **Not at risk but want protection from hepatitis B** (identification of risk factor not required): 2- or 3-dose series (2-dose series Heplisav-B at least 4 weeks apart [2-dose series HepB only applies when 2 doses of Heplisav-B are used at least 4 weeks apart] or 3-dose series Engerix-B or Recombivax HB at 0, 1, 6 months [minimum intervals: 4 weeks between doses 1 and 2, 8 weeks between doses 2 and 3, 16 weeks between doses 1 and 3]) or 3-dose series HepA-HepB (Twinrix at 0, 1, 6 months [minimum intervals: 4 weeks between doses 1 and 2, 5 months between doses 2 and 3])

Special situations

- **At risk for hepatitis B virus infection:** 2-dose (Heplisav-B) or 3-dose (Engerix-B, Recombivax HB) series or 3-dose series HepA-HepB (Twinrix) as above
  - **Chronic liver disease** (e.g., persons with hepatitis C, cirrhosis, fatty liver disease, alcoholic liver disease, autoimmune hepatitis, alanine aminotransferase [ALT] or aspartate aminotransferase [AST] level greater than twice upper limit of normal)
  - **HIV infection**

- **Sexual exposure risk** (e.g., sex partners of hepatitis B surface antigen [HBsAg]-positive persons; sexually active persons not in mutually monogamous relationships; persons seeking evaluation or treatment for a sexually transmitted infection; men who have sex with men)
- **Current or recent injection drug use**
- **Percutaneous or mucosal risk for exposure to blood** (e.g., household contacts of HBsAg-positive persons; residents and staff of facilities for developmentally disabled persons; health care and public safety personnel with reasonably anticipated risk for exposure to blood or blood-contaminated body fluids; hemodialysis, peritoneal dialysis, home dialysis, and predialysis patients; persons with diabetes mellitus age younger than 60 years and, at discretion of treating clinician, those age 60 years or older)
- **Incarcerated persons**
- **Travel in countries with high or intermediate endemic hepatitis B**
- **Pregnancy** if at risk for infection or severe outcome from infection during pregnancy. Heplisav-B not currently recommended due to lack of safety data in pregnant women

Human papillomavirus vaccination

Routine vaccination

- **HPV vaccination recommended for all adults through age 26 years:** 2- or 3-dose series depending on age at initial vaccination or condition:
  - **Age 15 years or older at initial vaccination:** 3-dose series at 0, 1–2, 6 months (minimum intervals: 4 weeks between doses 1 and 2/12 weeks between doses 2 and 3/5 months between doses 1 and 3; repeat dose if administered too soon)
  - **Age 9 through 14 years at initial vaccination and received 1 dose or 2 doses less than 5 months apart:** 1 dose
  - **Age 9 through 14 years at initial vaccination and received 2 doses at least 5 months apart:** HPV vaccination complete, no additional dose needed.
- **If completed valid vaccination series with any HPV vaccine, no additional doses needed**

Shared clinical decision-making

- **Age 27 through 45 years based on shared clinical decision-making:**
  - 2- or 3-dose series as above

Special situations

- **Pregnancy through age 26 years**: HPV vaccination not recommended until after pregnancy; no intervention needed if vaccinated while pregnant; pregnancy testing not needed before vaccination

Influenza vaccination

Routine vaccination

- **Persons age 6 months or older**: 1 dose any influenza vaccine appropriate for age and health status annually
- For additional guidance, see www.cdc.gov/flu/professionals/index.htm

Special situations

- **Egg allergy, hives only**: 1 dose any influenza vaccine appropriate for age and health status annually
- **Egg allergy more severe than hives** (e.g., angioedema, respiratory distress): 1 dose any influenza vaccine appropriate for age and health status annually in medical setting under supervision of health care provider who can recognize and manage severe allergic reactions
- **LAIV should not be used** in persons with the following conditions or situations:
  - History of severe allergic reaction to any vaccine component (excluding egg) or to a previous dose of any influenza vaccine
  - Immunocompromised due to any cause (including medications and HIV infection)
  - Anatomic or functional asplenia
  - Cochlear implant
  - Cerebrospinal fluid-oropharyngeal communication
  - Close contacts or caregivers of severely immunosuppressed persons who require a protected environment
  - Pregnancy
  - Received influenza antiviral medications within the previous 48 hours
- **History of Guillain-Barré syndrome within 6 weeks of previous dose of influenza vaccine**: Generally should not be vaccinated unless vaccination benefits outweigh risks for those at higher risk for severe complications from influenza

Measles, mumps, and rubella vaccination

Routine vaccination

- **No evidence of immunity to measles, mumps, or rubella**: 1 dose
  - **Evidence of immunity:** Born before 1957 (health care personnel, see below), documentation of receipt of MMR vaccine, laboratory, laboratory evidence of immunity or disease (diagnosis of disease without laboratory confirmation is not evidence of immunity)

Special situations

- **Pregnancy with no evidence of immunity to rubella**: MMR contraindicated during pregnancy; after pregnancy (before discharge from health care facility), 1 dose
- **Nonpregnant women of childbearing age with no evidence of immunity to rubella**: 1 dose
- **HIV infection with CD4 count ≥200 cells/μL for at least 6 months and no evidence of immunity to measles, mumps, or rubella**: 2-dose series at least 4 weeks apart; MMR contraindicated in HIV infection with CD4 count <200 cells/μL
- **Severe immunocompromising conditions**: MMR contraindicated
- **Students in postsecondary educational institutions, international travelers, and household or close,**

personal contacts of immunocompromised persons, with no evidence of immunity to measles, mumps, or rubella: 2-dose series at least 4 weeks apart if previously did not receive any MMR or 1 dose if previously received 1 dose MMR

- **Health care personnel:**
  - **Born in 1957 or later with no evidence of immunity to measles, mumps, or rubella**: 2-dose series at least 4 weeks apart for measles or mumps or at least 1 dose MMR for rubella
  - **Born before 1957 with no evidence of immunity to measles, mumps, or rubella**: Consider 2-dose series at least 4 weeks apart for measles or mumps or 1 dose for rubella

Meningococcal vaccination

Special situations for MenACWY

- **Anatomical or functional asplenia (including sickle cell disease), HIV infection, persistent complement component deficiency, complement inhibitor (e.g., eculizumab, ravulizumab) use**: 2-dose series MenACWY (Menactra, Menveo) at least 8 weeks apart and revaccinate every 5 years if risk remains
- **Travel in countries with hyperendemic or epidemic meningococcal disease, microbiologists routinely exposed to *Neisseria meningitidis***: 1 dose MenACWY (Menactra, Menveo) and revaccinate every 5 years if risk remains
- **First-year college students who live in residential housing (if not previously vaccinated at age 16 years or older) and military recruits**: 1 dose MenACWY (Menactra, Menveo)

Shared clinical decision-making for MenB

- **Adolescents and young adults age 16 through 23 years (age 16 through 18 years preferred) not at increased risk for meningococcal disease**: Based on shared clinical decision-making, 2-dose series MenB-4C at least 1 month apart, or 2-dose series MenB-FHbp at 0, 6 months (if dose 2 was administered less than 6 months after dose 1, administer dose 3 at least 4 months after dose 2); MenB-4C and MenB-FHbp are not interchangeable (use same product for all doses in series)

Special situations for MenB

- **Anatomical or functional asplenia (including sickle cell disease), persistent complement component deficiency, complement inhibitor (e.g., eculizumab, ravulizumab) use, microbiologists routinely exposed to *Neisseria meningitidis***: 2-dose primary series MenB-4C (Bexsero) at least 1 month apart, or 3-dose primary series MenB-FHbp (Trumenba) at 0, 1–2, 6 months (if dose 2 was administered at least 6 months after dose 1, dose 3 not needed); MenB-4C and MenB-FHbp are not interchangeable (use same product for all doses in series); 1 dose MenB booster 1 year after primary series and revaccinate every 2–3 years if risk remains
- **Pregnancy**: Delay MenB until after pregnancy unless at increased risk and vaccination benefits outweighs potential risks

Pneumococcal vaccination

Routine vaccination

- **Age 65 years or older** (immunocompetent):– see [New Pneumococcal Vaccine Recommendations for Adults Aged ≥65 Years Old]: 1 dose PPSV23
  - If PPSV23 was administered prior to age 65 years, adminster 1 dose PPSV23 at least 5 years after previous dose

Shared clinical decision-making

- **Age 65 years and older** (immunocompetent): 1 dose PCV13 based on **shared clinical decision-making**
  - If both PCV13 and PPSV23 are to be administered, PCV13 should be administered first
  - PCV13 and PPSV23 should be administered at least 1 year apart.
  - PCV13 and PPSV23 should not be administered during the same visit

Special situations see (New Pneumococcal Vaccine Recommendations for Adults Aged ≥65 Years Old)

- **Age 19 through 64 years with chronic medical conditions (chronic heart [excluding hypertension], lung, or liver disease, diabetes), alcoholism, or cigarette smoking**: 1 dose PPSV23
- **Age 19 years or older with immunocompromising conditions (congenital or acquired immunodeficiency [including B- and T-lymphocyte deficiency, complement deficiencies, phagocytic disorders, HIV infection], chronic renal failure, nephrotic syndrome, leukemia, lymphoma, Hodgkin disease, generalized malignancy, iatrogenic immunosuppression [e.g., drug or radiation therapy], solid organ transplant, multiple myeloma) or anatomical or functional asplenia (including sickle cell disease and other hemoglobinopathies)**: 1 dose PCV13 followed by 1 dose PPSV23 at least 8 weeks later, then another dose PPSV23 at least 5 years after previous PPSV23; at age 65 years or older, administer 1 dose PPSV23 at least 5 years after most recent PPSV23 (note: only 1 dose PPSV23 recommended at age 65 years or older)
- **Age 19 years or older with cerebrospinal fluid leak or cochlear implant**: 1 dose PCV13 followed by 1 dose PPSV23 at least 8 weeks later; at age 65 years or older, administer another dose PPSV23 at least 5 years after PPSV23 (note: only 1 dose PPSV23 recommended at age 65 years or older)

Tetanus, diphtheria, and pertussis vaccination

Routine vaccination

- **Previously did not receive Tdap at or after age 11 years**: 1 dose Tdap, then Td or Tdap every 10 years

Special situations

- **Previously did not receive primary vaccination series for tetanus, diphtheria, or pertussis**: At least 1 dose Tdap followed by 1 dose Td or Tdap at least 4 weeks after Tdap and another dose Td or Tdap 6–12 months after last Td or Tdap (Tdap can be substituted for any Td dose, but preferred as first dose); Td or Tdap every 10 years thereafter
- **Pregnancy**: 1 dose Tdap during each pregnancy, preferably in early part of gestational weeks 27–36
- For information on use of Td or Tdap as tetanus prophylaxis in wound management, see Prevention of Pertussis,

Tetanus, and Diphtheria with Vaccines in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP).

Varicella vaccination

Routine vaccination

- **No evidence of immunity to varicella**: 2-dose series 4–8 weeks apart if previously did not receive varicella-containing vaccine (VAR or MMRV [measles-mumps-rubella-varicella vaccine] for children); if previously received 1 dose varicella-containing vaccine, 1 dose at least 4 weeks after first dose
    - Evidence of immunity: U.S.-born before 1980 (except for pregnant women and health care personnel [see below]), documentation of 2 doses varicella-containing vaccine at least 4 weeks apart, diagnosis or verification of history of varicella or herpes zoster by a health care provider, laboratory evidence of immunity or disease

Special situations

- **Pregnancy with no evidence of immunity to varicella**: VAR contraindicated during pregnancy; after pregnancy (before discharge from health care facility), 1 dose if previously received 1 dose varicella-containing vaccine or dose 1 of 2-dose series (dose 2: 4–8 weeks later) if previously did not receive any varicella-containing vaccine, regardless of whether U.S.-born before 1980
- **Health care personnel with no evidence of immunity to varicella**: 1 dose if previously received 1 dose varicella-containing vaccine; 2-dose series 4–8 weeks apart if previously did not receive any varicella-containing vaccine, regardless of whether U.S.-born before 1980
- **HIV infection with CD4 count ≥200 cells/μL with no evidence of immunity**: Vaccination may be considered (2 doses, administered 3 months apart); VAR contraindicated in HIV infection with CD4 count <200 cells/μL
- **Severe immunocompromising conditions**: VAR contraindicated

Zoster vaccination

Routine vaccination

- **Age 50 years or older:** 2-dose series RZV (Shingrix) 2–6 months apart (minimum interval: 4 weeks; repeat dose if administered too soon) regardless of previous herpes zoster or history of ZVL (Zostavax) vaccination (administer RZV at least 2 months after ZVL)
- **Age 60 years or older**: 2-dose series RZV 2–6 months apart (minimum interval: 4 weeks; repeat if administered too soon) or 1 dose ZVL if not previously vaccinated. RZV preferred over ZVL (if previously received ZVL, administer RZV at least 2 months after ZVL)

Special situations

- **Pregnancy**: ZVL contraindicated; consider delaying RZV until after pregnancy if RZV is otherwise indicated
- **Severe immunocompromising conditions (including HIV infection with CD4 count <200 cells/μL)**: ZVL contraindicated; recommended use of RZV under review

Vaccines in the Adult Immunization Schedule

Vaccines	Abbreviations	Trade names
Haemophilus influenzae **type b**	Hib	ActHIB® Hiberix® PedvaxHIB®
Hepatitis A vaccine	HepA	Havrix® Vaqta®
Hepatitis A and hepatitis B vaccine	HepA-HepB	Twinrix®
Hepatitis B vaccine	HepB	Engerix-B® Recombivax HB® Heplisav-B®
Human papillomavirus vaccine	HPV vaccine	Gardasil 9®
Influenza vaccine, inactivated	IIV	Many brands
Influenza vaccine, live, attenuated	LAIV	FluMist® Quadrivalent
Influenza vaccine, recombinant	RIV	Flublok Quadrivalent®
Measles, mumps, and rubella vaccine	MMR	M-M-R® II
Meningococcal serogroups A, C, W, Y vaccine	MenACWY	Menactra® Menveo®
Meningococcal serogroup B vaccine	MenB-4C MenB-FHbp	Bexsero® Trumenba®
Pneumococcal 13-valent conjugate vaccine	PCV13	Prevnar 13®
Pneumococcal 23-valent polysaccharide vaccine	PPSV23	Pneumovax® 23
Tetanus and diphtheria toxoids	Td	Tenivac® Tdvax™
Tetanus and diphtheria toxoids and acellular pertussis vaccine	Tdap	Adacel® Boostrix®
Varicella vaccine	VAR	Varivax®

Zoster vaccine, recombinant	RZV	Shingrix
Zoster vaccine live	ZVL	Zostavax®

This schedule is recommended by the Advisory Committee on Immunization Practices (ACIP) and approved by the Centers for Disease Control and Prevention (CDC), American College of Physicians (ACP), American Academy of Family Physicians (AAFP), American College of Obstetricians and Gynecologists (ACOG), and American College of Nurse-Midwives (ACNM).

The comprehensive summary of the ACIP recommended changes made to the adult immunization schedule can be found in the February 6, 2020 *MMWR*.

Report

- Suspected cases of reportable vaccine-preventable diseases or outbreaks to the local or state health department
- Clinically significant postvaccination reactions to the Vaccine Adverse Event Reporting System or 800-822-7967

Injury Claims

- All vaccines included in the adult immunization schedule except pneumococcal 23-valent polysaccharide and zoster vaccines are covered by the Vaccine Injury Compensation Program. Information on how to file a vaccine injury claim is available at www.hrsa.gov/vaccinecompensation or 800-338-2382.
- Clinically significant postvaccination reactions to the Vaccine Adverse Event Reporting System or 800-822-7967

Helpful information

- Complete ACIP recommendations
- General Best Practice Guidelines for Immunization
- Vaccine Information Statements
- Manual for the Surveillance of Vaccine-Preventable Diseases (including case identification and outbreak response)
- Travel vaccine recommendations

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Content source: National Center for Immunization and Respiratory Diseases

EXHIBIT 347

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Immunization Schedules

Schedule-Related Resources

CDC offers resources to help you learn about the immunization schedules, including ACIP immunization recommendations, schedule presentation graphics, and past immunization schedules. CDC also encourages you to share the immunization schedule and the importance of timely vaccination through your website and social media channels.

ACIP immunization recommendations +

Schedule presentation graphics +

Prior immunization schedules −

See how the schedules have changed over the years.

Prior Years' Child/Adolescent Immunization Schedules

- **2019**
  - Table 1. By age
  - Table 2. Catch-up
  - Table 3. By medical indications
  - PDF [8 pages]
- 2018 [8 pages]
- 2017 [8 pages]
- 2016 [6 pages]
- 2015 [6 pages]
- 2014
  - Schedule [6 pages]
  - Changes
- 2013
- 2012
- 2011
- 2010
- 2009
- 2008
- 2007

- 2006
- 2005
- 2004 (Jan-Jun)
- 2004 (Jul-Dec)
- 2003
- 2002
- 2001
- 2000
- 1999
- 1998
- 1997
- 1996
- 1995
- 1994(.jpg)
- 1989(.jpg)
- 1983(.jpg)

See also:

- History of Vaccination Schedule
  Children's Hospital of Philadelphia's Vaccine Education Center

Prior Years' Adult Immunization Schedules

- 2019
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[From The Merck Manual, Eighth Edition, published 1950]

ROUTINE IMMUNIZATION PROCEDURES

Optional pediatric immunization schedules and timetables for the administration of booster or re-immunization doses are presented. A table outlining the use of human serum immune (gamma) globulin also is included. Although many pertinent details are given, actual dosage must be regulated according to individual circumstances and to the instructions accompanying packages of the various immunizing agents. (For special immunization procedures against such diseases as typhoid fever, yellow fever, cholera, plague, and other conditions not ordinarily included in pediatric practice, *see* the respective chapters.)

BASIC IMMUNIZATION

OPTIONAL SCHEDULE NO. 1

Age	Agent
3 months	Pertussis Vaccine (Alum Precipitated)
4 "	" " " "
5 "	" " " "
6 "	Diphtheria-Tetanus Toxoid (Alum Precipitated)
6 "	Smallpox Vaccine
7 "	Diphtheria-Tetanus Toxoid (Alum Precipitated)
11 "	Schick Test
11 "	Pertussis Vaccine (Alum Precipitated)

OPTIONAL SCHEDULE NO. 2

Age	Agent
As soon as umbilicus is healed and baby is thriving	Smallpox Vaccine
3 months	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
4 "	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
5 "	Pertussis Vaccine (Alum Precipitated)
7 "	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
11 "	Schick Test

BOOSTER DOSES AND RE-IMMUNIZATION

(This schedule applies only when basic immunization has been previously accomplished.)

Age and Indication	Agent
2 years	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
5 "	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
5 "	Schick Test
Every 2 years	Smallpox Vaccine
Every 5 years or upon exposure to smallpox, or during threatened smallpox epidemic	Tetanus Toxoid (Alum Precipitated)
Any age, upon possible exposure to tetanus	Smallpox Vaccine
Any age, upon exposure to diphtheria	Fluid Tetanus Toxoid
Any age, upon exposure to pertussis	Fluid Diphtheria Toxoid
	Pertussis Vaccine (N.B., in Isotonic Saline)

EXHIBIT 349

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Immunization practice in the United States and Great Britain: a comparative study

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'Epidemics that used to be excused as acts of God are now not excused as the results of the inactivity of man. In short, the incidence of many diseases has been moved from the area of chance to the area of choice. That is a vast change intellectually. Not only intellectually but also morally, for such a series of accomplishments leaves us with a new system of ethics to devise, somewhat as the perfection of the automobile has called for new traffic laws. As physicians we cannot evade a moral responsibility that goes with our newly acquired power. Having learned how disease comes about, we find ourselves answerable for why it should occur at all' (Gregg, 1949).

THE EFFECTIVE control or eradication of many infectious diseases through prophylactic immunization stands as one of the more dramatic success stories of modern medicine. The number of diseases amenable to prevention is increasing in recent years, and new developments on the horizon may be expected to further compound the already complicated immunization schedule. We have arrived at the point where, from infancy through young adulthood, the individual will receive a formidable array of biological products for the prevention of disease on a *routine* basis. Decisions concerning the optimal use of immunizing agents have attained a high degree of sophistication, stemming from a number of very real factors. Expert opinion has been divided on many issues. Matters of fine judgment and public policy are involved. Thus, the individual practitioner or public health officer finds that he must become learned in such areas as: the relative merits of inactivated *versus* live attenuated virus vaccines; the effect of adjuvants; the optimal sequence and interval of administration; the efficiency and safety of available products; the identification of high risk groups to be singled out for special immunization schedules; the contraindications to administration of individual products; the attributes of newly-introduced biologicals and weighing of risk of disease against the risk of the immunizing procedure; the legitimacy of universal immunization against a disease which is absent or uncommon in the population; and the problems of simultaneous administration of immunizing agents, especially one or more live products.

Final judgment on the safest and most effective procedures to use for the immunization of the individual, community or nation rests on a complex balance of factors. More and more, the individual practitioner and the public health authorities have looked for guidance from expert national bodies. This guidance in recent years has been based increasingly on pre-designed studies such as vaccine field trials, surveillance of disease morbidity and mortality, sero-epidemiology, and surveillance of the untoward effects of vaccines. These data are then weighed against the practicalities of scheduling such immunizations within the existing patterns of delivery of health care.

The present article is an attempt to explore some of the problems and solutions undertaken in the field of immunization practice in the United States and Great Britain. A very limited review in the two countries has shown that, despite the disparate systems of delivery of health care, similar trends in immunization practice have developed. Furthermore, the rate of use of various products and their effectiveness in reduction of disease again shows more resemblances than differences. One conspicuous parallel development in the United States and Great Britain has been the increasing emergence of unified national policy concerning the use of biological reagents for immunoprophylaxis. The recommendations of national bodies, representing the consensus of informed opinion, have been powerful forces in the use of immunizing agents. These developments can be briefly traced in each country.

In the United States, one of the early influences has been the Committee on the Control of Infectious Diseases, a standing committee of the American Academy of Pediatrics. The first edition of their report, covering eight pages, was published in 1938. The current fifteenth edition, published in 1966, is a 185-page desk reference which reviews recommenda-

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tions concerning prevention of most of the infectious diseases encountered in North and South America (American Academy of Pediatrics, 1966). The report has been translated into Spanish. Through the years, the red-covered report, referred to as 'The Red Book', has become the veritable bible for the practitioner caring for children, as well as a reference point for public health authorities and well-baby clinics

During the early years of the introduction of poliovirus and measles vaccines, the United States Public Health Service issued recommendations through expert committees assembled on an *ad hoc* basis. To meet the need for evaluation of new and old vaccines on a continuing basis, the Advisory Committee on Immunization Practices was created in 1964. This committee was charged with apprising the Surgeon-General of the status of diseases for which effective vaccines are available, and to advise regularly on immunization practices relevant to these diseases in public health and preventive medical practice in the United States. The committee was further charged with encouraging investigation of vaccine usage and disease surveillance. Since its inception, the committee has issued formal statements on most of the major vaccines, updating these statements as new information emerged. The Advisory Committee relies heavily on the staff of the National Communicable Disease Center (NCDC) for program assistance. The NCDC has recently published a volume, *Immunization Against Diseases*, 1966–67 (United States Public Health Service, 1967), which includes current epidemiological reviews of selected infectious diseases, a summary of immunization status and use of biologicals in the United States, and the complete reports of the Advisory Committee recommendations.

Another potent factor in increasing the utilization of vaccines in the United States has been the trend at the state level to require certain immunizations prior to school entry. Since February 1968, half of the states required immunization against one or more diseases, including twelve which require measles, sixteen poliovirus, thirteen diphtheria–tetanus–pertussis and twenty-one smallpox vaccines (United States Public Health Service, 1968a). These compulsory immunization laws are prosecuted with various degrees of conviction in different parts of the country, although exemptions are usually granted when immunizations are contrary to religious beliefs or medically contraindicated. It would appear that the trend to increasing compulsory immunization will continue, insuring that children are adequately immunized in this captive manner when entering this critical time of increased risk.

In 1962, Congress passed the Vaccination Assistance Act, which provided financial assistance to state and local health departments for improvement of immunization programs. Funds were made available for purchase of vaccines for immunization of pre-school children, support of the organization and administration of immunization programs, improvement of laboratory and epidemiological surveillance, and promotional and educational activities. Although the bulk of the support has emphasized public health and community-based programs, it has been observed in most areas that the balance between immunizations given privately and publicly does not change when a community accelerates its immunization activities (Freckleton, 1967). Appropriations for the program have ranged from 8 to 10 million dollars a year. At present there are forty-eight state and twenty-four local grant-assisted programs, covering approximately 90% of the population of the United States, Puerto Rico and the Virgin Islands. By design, the program has permitted a high degree of flexibility of administration to adapt to local needs.

Another dimension that bears on the final efficiency of immunization programs has become evident in the United States. There are wide differences in the rates of immunization in various demographic groups in the population. For example, vaccine acceptance has been particularly poor in the poverty areas of the large cities in the United States, especially among the Negro population. This has also been true in certain rural regions where poverty and other special cultural factors may prevail. Such under-immunized groups often have a higher incidence of preventable diseases and in turn have been the target for intensive immunization campaigns. Specific examples of this phenomenon will be pointed out under individual diseases.

In Great Britain, over the past 20 years, there has been increasing guidance concerning immunization policy from the Ministry of Health. In 1948, when the National Health Service Act was established, smallpox vaccination and diphtheria immunization were the only officially recommended procedures in England and Wales (Ministry of Health, 1964). Pertussis immunization was undertaken by some local health authorities. BCG vaccination was later introduced to school children on a discretionary basis. Various alternative schemes for immunization with diphtheria, pertussis and tetanus were recommended in 1954 (Ministry of Health, 1955). Official schedules of combined vaccines were prepared in 1961 and adopted by local health authorities. Special committees dealing with matters such as diphtheria toxoid and poliomyelitis vaccine were superceded in 1962 by a Joint Committee on Vaccination and Immunization appointed by the Central Health Services Council and the Ministry of Health, to 'advise the Health Ministers on all the medical

Exhibit 349

TABLE 1. Recommended schedules for routine immunization

United States*					England and Wales†					
Age	DTP	OPV	M	SP	Age	DTP	OPV	M	SP	BCG
2–3 months	X	X			3–6 months	X	X			
3–4 months	X									
4–5 months	X	X			5–8 months	X	X			
12–18 months	X	X	X		9–14 months	X	X			
12–24 months				X	12–24 months			X	X	
School entry (3–6 years)	X	X		X	School entry (3–6 years)	Td	X		X	
					10–13 years					X
Every 10 years	Td			X‡	School leaving	Td	X		X	

DTP, Diphtheria–tetanus–pertussis vaccine; OPV, oral poliovaccine; M, measles vaccine; SP, smallpox vaccine; Td, tetanus-diphtheria toxoid, adult type.

* Adopted from United States Public Health Service (1967): *Immunization Against Disease* 1966–67 (National Communicable Disease Center publication).

† Adopted from Ministry of Health (1968a,b).

‡ For high risk groups, i.e. health personnel and overseas travel—every 3 years.

aspects of vaccination and immunization'. Suggested schedules were published in 1963 in a booklet entitled *Active Immunization Against Infectious Disease* (Ministry of Health, 1963). This Joint Committee has the same general mission as the United States Public Health Service Advisory Committee on Immunization Practices.

Schedules for those immunization procedures recommended for all children are shown in Table 1. The table has been constructed using the most recent statements available from the United States Public Health Service and the Ministry of Health (United States Public Health Service, 1967; Ministry of Health, 1963, 1968a). It is notable that the schedules are remarkably similar, despite the fact that, historically, there have been differences in the approach to the use of individual vaccines. There are several points of difference. DTP combined antigen is recommended at an earlier age in the United States, and three doses are administered in the primary series to make up for the handicaps of immaturity and maternal antibody. In the schedule recently recommended for Great Britain, initiation of immunization at approximately 6 months is favoured. This is an effort to avoid the immunological handicap and also to lessen the risk of reactions to pertussis vaccine thought to be more common in children under 6 months. A booster dose of pertussis vaccine is included in the United States schedule because of the continued threat of disease beyond infancy and the apparently more benign experience with reactions. BCG is not used routinely in school-aged children in the United States, but is reserved for selected individuals considered to be at high risk. An additional dose of oral poliovaccine recommended at school-leaving in Great Britain is thought to be unnecessary in the United States.

Status of individual diseases

Certain diseases for which immunoprophylaxis is available will be discussed in further detail. Information concerning immunization rates, morbidity and mortality, and other problems related to vaccine use, have been gathered from a variety of sources. Except when noted, all British data refer to England and Wales. In looking at the statistical data, it is useful to remember that the population of the United States, 196 million, is approximately four times that of England and Wales, 48 million (1966). In attempting to assemble comparable data for the United States and Great Britain, it was immediately evident that a strict comparison of the use of vaccine or disease incidence was not possible because of inherent differences in notification of disease, surveillance methods, and book-keeping procedures. Despite this, many instructive comparisons can be drawn between practices in the two countries.

Diphtheria, tetanus and pertussis

The three diseases may be discussed profitably under one heading, because of the current general practice of immunization with combined antigens. Table 2 shows the percentage of children in the United States who had completed a primary course in DTP in 1966. Approximately 83% of school-aged children have received at least three, and 65% have received four or more, doses of vaccine. The difference in rates between white and non-white populations is striking at all ages, but is most marked in the younger age groups. Immunization rates for a primary series of diphtheria and pertussis in England and Wales were similar to those in the United States for the first 2 years of life. The somewhat lower percentages of children under 5 or under 16 who were

TABLE 2. Immunization rate (%), combined diphtheria and tetanus toxoids and pertussis vaccine (1966)

UNITED STATES

Age (years)	Diphtheria–tetanus–pertussis*		
	Total	White	Non-white
1	69	74	44
2	74	78	52
3	76	79	56
4	79	82	60
1–4	74	79	53
5–9	83	86	70

ENGLAND AND WALES

Age (years)	Diphtheria	Pertussis
1†	73	72
2†	76	74
Under 5‡	68	
Under 16‡	52	

* Three or more doses of vaccine (United States Public Health Service, 1967).

† Primary series (Ministry of Health, 1967a).

‡ Percentage of children under 5 years or 16 years that 'may be regarded as remaining protected against diphtheria) (Ministry of Health, 1967a).

regarded as protected against diphtheria would represent children who had not received appropriately timed booster doses. Data were not available to the author regarding rates of tetanus immunization in England and Wales, but the magnitude can be assumed to be of the same order as that for diphtheria and pertussis. Immunization rates are far from uniformly distributed within each country. For example, while 74% of the children between 1 and 4 years of age received DTP in the United States, sections of the country varied considerably in their rates. In the New England region, 81%, and in the west south-central region, 66% had received three or more doses of DTP. Rates of 91 and 92% for both diphtheria and pertussis immunization in the 1st and 2nd years of life, respectively, attained in West Sussex, were well above the general rates for England. West Sussex has instituted an automatic data-processing procedure to permit an intensive follow-up of all infants (Ministry of Health, 1967a).

There are several factors which historically have delayed the enthusiastic endorsement of combined triple antigen early in infancy, especially in Great Britain. The use of DTP received a temporary setback as a result of reports such as that prepared by the Medical Research Council in 1956, which drew attention to the increased risk of provoking poliomyelitis with such injections (Ministry of Health, 1964). The virtual disappearance of poliovirus from the community largely discounted this objection. However, until recently, it was recommended that triple antigen be separated from oral polio-vaccine by 3 weeks (Ministry of Health, 1963). This in itself has complicated the orderly scheduling of immunization and required an increased number of visits. Concern has been expressed regarding the number of important reactions (e.g. shock, neurological damage) accompanying the pertussis component of the combined antigen, and this type of event is said to occur more frequently under the age of 6 months. Similar alarming suggestions concerning the high rate of neurological complications in Sweden have been reported (Ström, 1960). In the United States, reactions to triple antigen administered in the first 6 months of life as a routine measure have resulted in very few documented instances of neurological damage since 1952, when a ceiling was placed upon the antigenic content of pertussis vaccine by the United States Public Health Service (Edsall, 1961). The suggestion has been made that the prevalent antigenic strains of *Bordetella pertussis* have changed in the past few years, and that recent vaccine breakthroughs in England are largely associated with serotypes not represented in the

TABLE 3. Reported cases and deaths from diphtheria, tetanus and pertussis (5 years, 1962–66)

	Cases/year	Deaths/year	Case fatality*	Annual rates/million	
				Cases	Deaths
United States†					
Diphtheria	285	36	13	1·5	0·19
Tetanus	294	196	67	1·5	1·0
Pertussis	12,481	86	0·69	64	0·44
England and Wales§					
Diphtheria	23	1·8	13	·47	0·04
Tetanus		28‡			
Pertussis	21,409	30	0·14	443	0·61

* Deaths per 100 reported cases.

† From United States Public Health Service (1967).

‡ Tetanus is not reportable in England and Wales, therefore this figure probably underestimated the true incidence. (Ministry of Health, 1967a).

§ From Ministry of Health (1967a).

vaccine (Preston, 1965). To date, there are no similar reports of antigenic shift in the United States.

The average number of reported cases and deaths from diphtheria, tetanus and pertussis for the 5-year period 1962 to 1966 is recorded in Table 3. It can be seen that the number of diphtheria cases and deaths annually per million population is somewhat higher in the United States than in England and Wales. For pertussis, the death-rate is approximately the same, although the number of reported cases appears to be relatively lower in the United States. Because the number of deaths is probably a more reliable estimate of the true incidence of disease, and there is no obvious reason to expect a different death-to-case ratio, one could postulate that there is a greater under-reporting of pertussis cases in the United States. Under-reporting of a common, frequently mild and often undiagnosed disease is not surprising for the United States, and this phenomenon appears again in the discussion of measles (see below). Fig. 1 illustrates the decline in morbidity and mortality for diphtheria in the United States from 1920 onward (United States Public Health Service, 1967). The case fatality, however, has not similarly yielded to modern therapy. The historical data on diphtheria for England and Wales available to the author would indicate a similar pattern. Fig. 2 illustrates the geographic distribution of diphtheria cases in the United States in 1966 (United States Public Health Service, 1967). Although general in distribution, the majority of the 209 cases occurred in the south-eastern states and affected primarily unimmunized segments of the population. In urban as well as rural areas, diphtheria was reported generally in lower socio-economic groups. The epidemic in Rosebud County occurred in an Indian population.

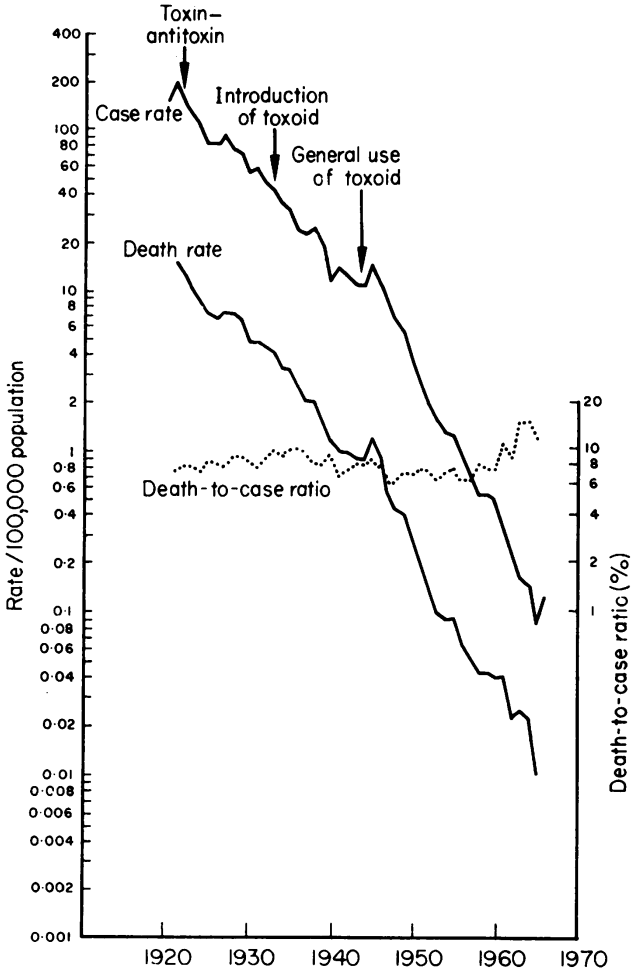

FIG. 1. Morbidity and mortality due to diphtheria in the United States, 1920–66 (United States Public Health Service, 1967).

Poliovaccine

The rates of immunization with poliovaccine are shown in Table 4. Although the criteria for a primary course of vaccine are somewhat different, the overall rates are quite comparable. In the United States, in the age group 1–4 years, approximately 70% have had either three doses of oral or inactivated vaccine and 79% two doses of oral poliovaccine. It is notable that the immunization rates are different in the white and non-white populations, reflecting the socio-economic differential in the distribution and/or acceptance of vaccine. The overall rates in England and Wales for the early years of life are very close to the United States data. The shift away from the use of inactivated poliovaccine is of interest. In 1966, less than 1% of the vaccine distributed in England and Wales was the inactivated type; in the United States, the figure was 17%, compared to 20% in 1965.

TABLE 4. Immunization with poliovaccine (1966)

Age (years)		3-OPV or 3-IPV	2-OPV and 0 to 2 IPV
United States*			
1–4	White	73	82
	Non-white	57	65
	Total	70	79
England and Wales†			
1		68	
2		72	
3		76	

OPV, Oral poliovaccine; IPV, inactivated poliovaccine.
* From United States Public Health Service (1967).
† From Ministry of Health (1967a,b).

Fig. 3 (United States Public Health Service, 1967) and Fig. 4 (Miller & Galbraith, 1965) show the dramatic decline in the incidence of poliomyelitis. In 1966, there were only nineteen cases of paralytic disease in England and Wales, a record low. The cases were sporadic, and no epidemic foci were reported. In the same year, 108 cases of paralytic disease occurred in the United States, two-thirds of

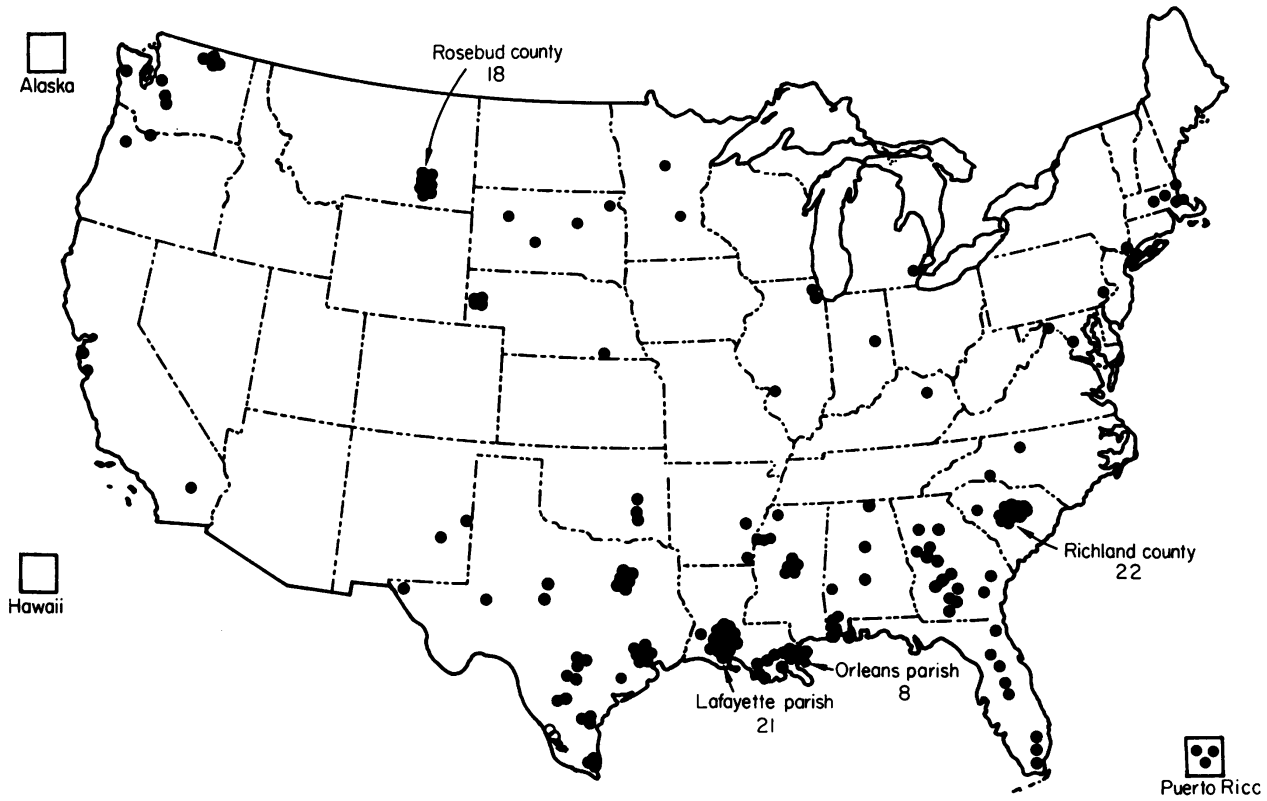

FIG. 2. Geographic distribution of reported cases (●) of diphtheria in the United States, 1966 (United States Public Health Service, 1967).

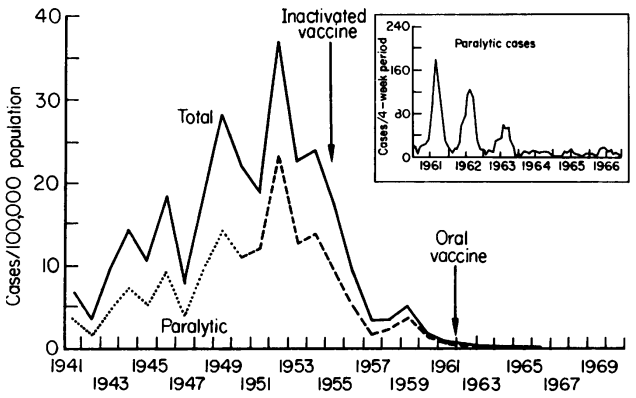

FIG. 3. Total poliomyelitis and paralytic cases reported in the United States, 1941–66 (United States Public Health Service, 1967).

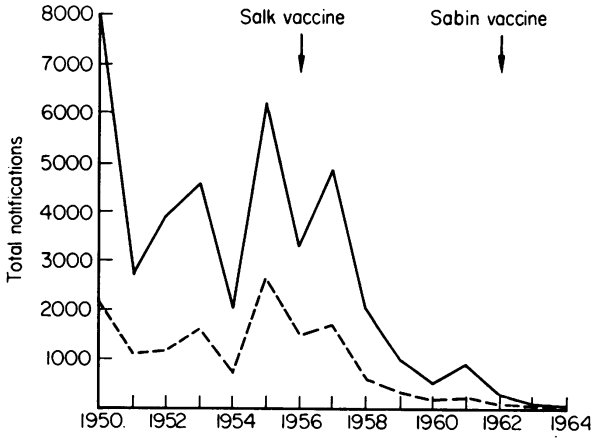

FIG. 4. Total poliomyelitis and paralytic cases reported in England and Wales, 1950–64 (Miller & Galbraith, 1965) —, Total notifications; . . . , non-paralytic cases.

which occurred in the state of Texas, where a type 1 poliovirus epidemic occurred (Fig. 5). The cases were concentrated primarily along the Mexican border. Seventy-five per cent of the paralytic cases had received no poliovaccine, and only seven cases had histories of adequate immunization. Surveillance in the United States since licensing of poliovaccines has shown that no more than one case of 'vaccine-related' paralytic disease has occurred for every 3 million does of oral poliovaccine administered, and these have occurred largely in adult males (United States Public Health Service, 1967). In 1966, there were ten possible 'vaccine-related' cases of poliomyelitis reported in the United States. One was related to the administration of inactivated poliovaccine, the other nine to oral vaccine, on the basis of the epidemiological criteria used. A similar study in England and Wales for the 3 years 1962 to 1964 revealed four cases with residual paralysis occurring between 5 and 28 days after vaccination, an incidence of one case in 4·5 million doses of live vaccine (Miller & Galbraith, 1965). Weighing the benefits of poliovaccine against the minute risks involved, its routine use appears to be well justified.

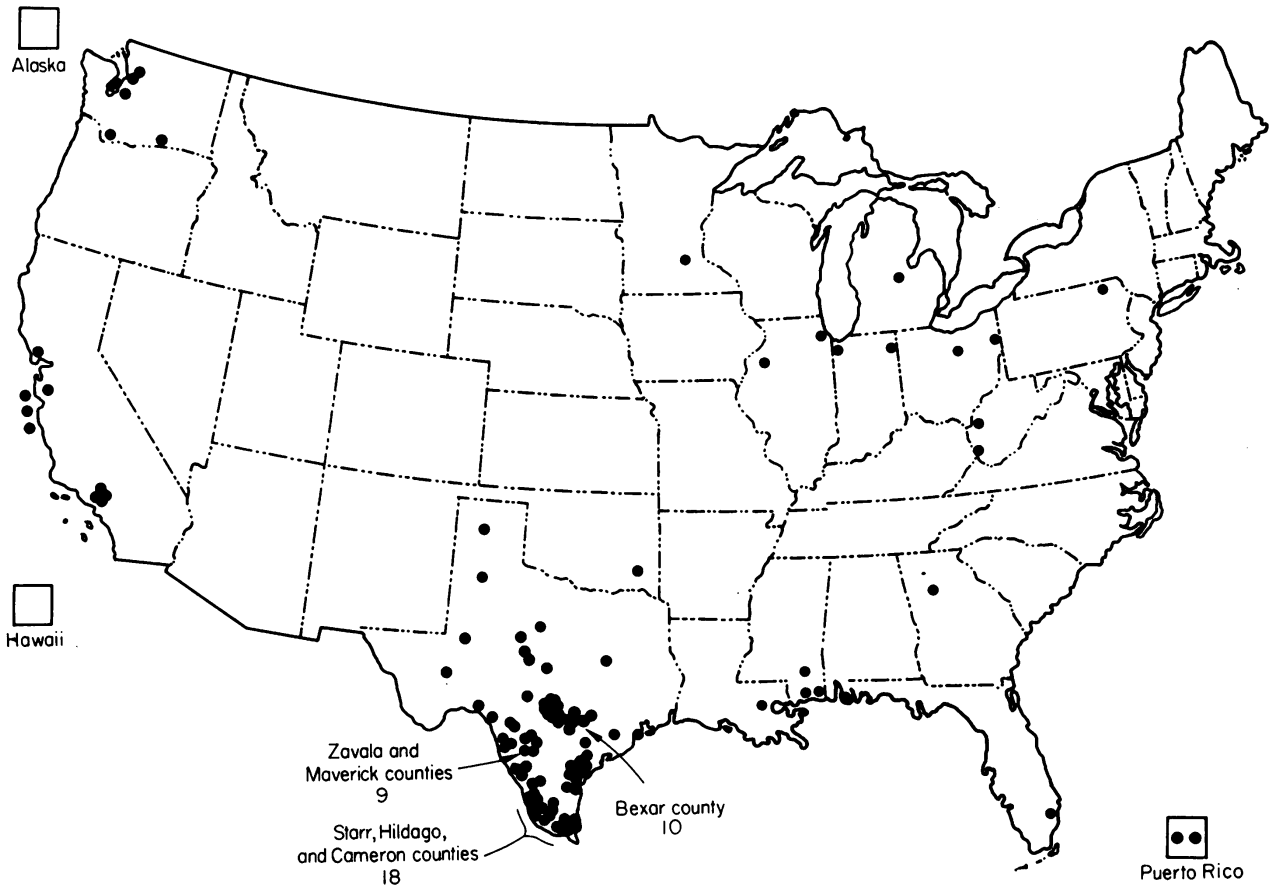

FIG. 5. Geographic distribution of reported paralytic poliomyelitis cases (●) in the United States, 1966 (United States Public Health Service, 1967).

TABLE 5. Immunization with smallpox vaccine

		U.S.*	England and Wales†		
		1964	1964	1965	1966
Total immunizations (thousands)	Primary	5420	340	390	480
	Revaccination	7370			70
	Unknown history	560			
Per cent infants immunized 0–2 years		57	32	33	38

* United States Public Health Service (1967).
† Ministry of Health (1967a).

Smallpox vaccine

Infants were immunized with smallpox vaccine at the rate of 32–38% in England and Wales in the years 1964–66. In comparison, the rate in the United States in 1964 was 57% (Table 5). Fig. 6 shows the smallpox immunization status of the population in the United States. A rate of 80% was attained by school entry, and over 95% of the population eventually received a primary vaccination (United States Public Health Service, 1967). In England and Wales, the 480,000 individuals who received primary smallpox immunization in 1966 were equivalent in number to slightly more than one-half the births for the year. In the United States in 1964, 5·4 million primary vaccinations were given, which is more than one and one-half times the number of births. In England and Wales, there were approximately 70,000 persons (1966) revaccinated, compared to 7·4 million revaccinations in the United States (1964). In the 10-year period 1951–60 in England and Wales, there was an average of 124,000 revaccinations per year. In recent years in the United States, there have been approximately 3 million individuals who have travelled overseas and received vaccinations on this account. Most of these are revaccinations. Thus, while all the data which

FIG. 6. Smallpox immunization status in the United States, surveyed in 1964 (United States Public Health Service, 1967). —, % vaccinated during lifetime; . . . , % vaccinated within past 12 months.

would be desirable are not at hand, indications are that the population of the United States is more highly vaccinated and revaccinated than that in England and Wales. However, in neither case are the rates sufficiently high to have a dependable herd immunity in face of an introduction of a case of smallpox.

In 1962, the Joint Committee on Vaccination and Immunization recommended that vaccination be performed optimally during the 2nd year of life, rather than at 4–5 months, as previously practiced (Ministry of Health, 1964). Further, it was urged that smallpox immunization be universally adopted, because it was clear that the vaccination rate for smallpox was well below that of other regular immunization procedures in infancy.

In the 10-year period 1957–66, in England and Wales, there were 142 cases of smallpox, with twenty-nine deaths, a mortality rate of 20·4% (Table 6) (Ministry of Health, 1967b). These resulted from eleven documented importations. In

TABLE 6. Smallpox in England and Wales, 1957–66*

Year	Known importations	Cases	Deaths
1957	1	4	2
1958	1	6	1
1959		1	
1960	1	1	
1961	2	2	
1962	6	66	26
1963–65			
1966		62	
Totals	11	142	29

* Ministry of Health (1967b)

1966, there were four outbreaks of *Variola minor*. The origins of these apparently multi-centered occurrences were not determined. Between 1961 and 1964, there were eleven instances of importation of smallpox into Great Britain, Germany, Sweden and Poland, resulting subsequently in a total of 222 cases and thirty-nine deaths before effective control was achieved (Karzon & Henderson, 1966). In many of the introductions, half or more of the subsequent cases were in hospital personnel.

In the United States, the last major outbreak occurred in 1947 in New York City, from a case imported from Mexico. The last potential introduction occurred as recently as 1962, when a 12-year-old boy from Brazil was admitted into the country at Kennedy Airport in New York. He departed immediately by train for Canada, where he became ill with typical smallpox shortly after arrival. Early diagnosis of the case, effective control measures, and an element of luck prevented the development of secondary cases (Karzon & Henderson, 1966).

Deaths, as well as central nervous and dermal complications of smallpox vaccination, in various age groups are shown in Table 7. The data from

TABLE 7. Complications associated with primary smallpox vaccination

Age (years)	Illness/million primary vaccinations			
	United States*		England and Wales†	
	Encephalitis	Dermal	Encephalitis	Dermal
1	1·5	145	15	47
1–4	0·7	41	6·2	27
5–14	3·7	34	30	34
15†	2·3	48	15	64
Overall rates	1·9	50	15	46
Totals				
Illness‡	12	318	56	174
Death	5	0	19	11
Rate/million vaccinations				
Illness	51		60	
Death	0·8		7·9	

* 6,240,000 primary vaccinations (1963) (Neff *et al.*, 1967b).
† 3,820,000 primary vaccinations (1951–60) (Conybeare, 1964).
‡ Illness includes deaths.

TABLE 8. Comparative rates of complications associated with primary smallpox vaccination or revaccination (per 1,000,000 vaccinated)

	United States		England and Wales‡
	U.S.*	Four States†	
Primary vaccination			
Illness	51	134	61
Death	0·8	0	7·9
Revaccination			
Illness	2·2	11	16
Death	0	0	2·4

* 6,240,000 primary and 7,780,000 revaccinations (1963) (Neff *et al.*, 1967a).
† 298,000 primary and 370,000 revaccinations (1963). An intensive survey which reflects increased frequency of minor dermal complications (Neff *et al.*, 1967b).
‡ 3,820,000 primary and 1,240,000 revaccinations (1951–60) (Conybeare, 1964).

England and Wales was reported routinely to the health authorities (Conybeare, 1964); the United States data stemmed largely from the list of patients for whom vaccinia immune-globulin had been requested, death certificates, routine reports to state health departments, and a national physician questionnaire (Neff *et al.*, 1967a, b). Based on this crude information, the incidence of encephalitis and the overall death rate were higher in England and Wales than in the United States. However, the rate of various types of dermal complications was approximately equal. Theoretically, half to two-thirds of the complications such as *Eczema vaccinatum* and *Vaccinia necrosum* may be prevented by more careful screening for known contraindications, and by delaying vaccination until after the 1st year of life. Contraindications include patients with depressed immune-response, either spontaneous or drug-induced; pregnancy; simultaneously administered live vaccines; and skin diseases, such as eczema, in the recipient or household contact. In general, there were approximately one death per million primary vaccinations in the United States, and fifty-three total complications per million. There were no deaths and 2·2 complications per million among 7·8 million revaccinees. In England, the death rate was somewhat less than eight per million, and the complications rate about sixty-one per million. There were 2·4 deaths and sixteen complications per million after revaccination. Thus, in both countries, revaccination was accompanied by a very small risk compared to primary vaccination (Table 8).

In another study, physicians in four states were surveyed by questionnaire concerning major and minor complications associated with smallpox vaccination. A significantly higher rate of *Eczema vaccinatum* and generalized vaccinia of a mild nature was uncovered. A total of 134 complications was reported per one million primary vaccinations and eleven per million revaccinations. Of the reported 279 patients with available data, only twenty-two were hospitalized (Neff *et al.*, 1967b) (Table 8).

The incidence and mortality from central nervous complications in both England and the United States is clearly less than that reported from certain other countries. In several studies reported from the continent, rates as high as one case in 1000 primary young adult vaccinees have been documented. There is no satisfactory explanation for this significant variation in incidence, although it should be noted that different strains of vaccinia virus are employed in various countries. The recommendation to defer immunization to the second year was based upon studies of Conybeare (1964), in which it was shown that encephalitis as well as dermal complications are far more frequent under 1 year of age than in the second year of life. Similar studies in the United States have also shown an excessive risk in children under 1 year of age (Table 7) (Neff *et al.*, 1967b).

In the United States, official policy has always been rather firm in advocating universal smallpox immunization, although this policy has been increasingly questioned (Dixon, 1962; Dick, 1962; Kempe & Benenson, 1965). Despite the requirement for a valid vaccination certificate, the possibility of introduction of a case into the United States is a real one. This risk is constantly changing, with increasing travel, but with a decreasing reservoir of endemic smallpox. Immunization to the fullest possible extent of infants and pre-school children is a principal means for community protection against introduced smallpox. Successfully vaccinated children should be essentially wholly immune for perhaps 3–5 years, with decreasing protection for a longer period. With primary sensitization accomplished, revaccination of older children and adults is attended by a much lower frequency of complications. Should a case of smallpox be introduced and recognized, subsequent control is accomplished by quarantine and selective vaccination; mass vaccination is considered inadvisable. Partial immunity of the popula-

tion as a result of previous vaccination offers certain advantages, in that the likelihood of spread in the community is diminished, and the antibody response to revaccination is more rapid and attains a higher titer (Ministry of Health, 1963). The value of chemoprophylaxis (methisazone) as an aid in epidemic control is not established, and the use of the drug is accompanied by a significant incidence of toxicity.

Measles vaccine

Live attenuated measles virus vaccine became available in the United States in 1963, and more than 30 million doses were distributed by the beginning of 1968. In late 1966, an intensive program was initiated, supported largely by the Vaccine Assistance Act, to eradicate measles in the United States. This was conducted through regular public and private immunization channels as well as through special community campaigns, the latter especially in areas with low immunization rates. Also, epidemics have been effectively aborted by the prompt administration of measles vaccine to selected groups of children, especially susceptibles in nursery school, kindergarden, and the first two or three grades of elementary school. The effectiveness of the total national effort is demonstrated in Fig. 7

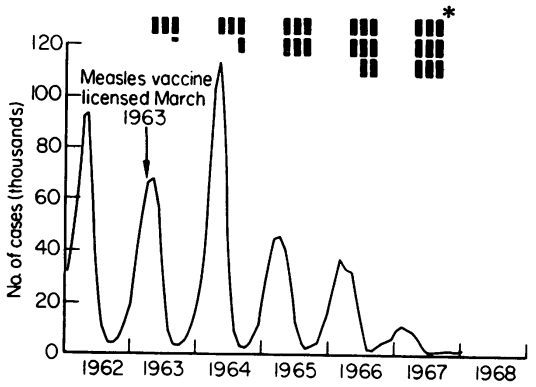

FIG. 7. Reported cases of measles in the United States, 1962–67, and distribution of live measles vaccine (United States Public Health Service, 1968a). Blocks, 1 million doses. *Estimated total for 1967.

(United States Public Health Service, 1968a). The number of reported cases of measles in 1967–68 is lower than any year since the onset of measles reporting in 1912, and is less than 10% of the expected incidence. The long-term trends are shown in Fig. 8.

A high percentage of pre-school children have now been immunized. In areas where special campaigns have been conducted, the rates are very high; for example, 94% in Los Angeles County and 91% in the state of Rhode Island. Other states reported between 50 and 80% of the pre-school population immunized at the end of 1967 (United

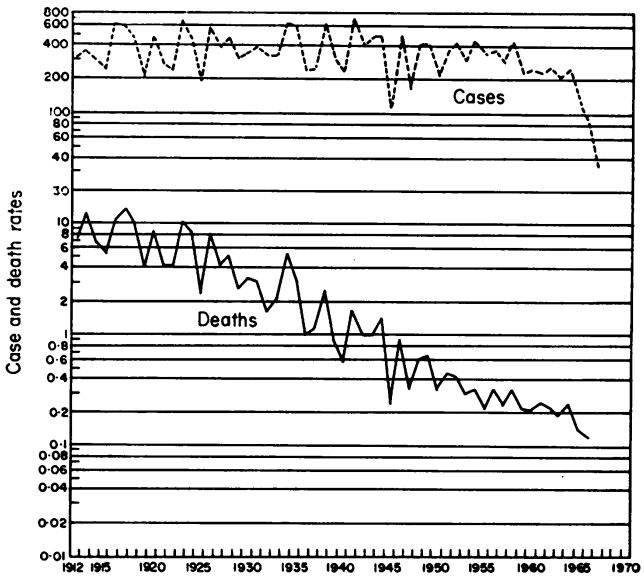

FIG. 8. Reported measles cases and deaths in the United States, 1912–67. Live measles vaccine was licensed in 1963 (United States Public Health Service, 1968c).

States Public Health Service, 1968a). Approximately thirty states report rates of 90% or more in school-aged children, a direct result of school-wide programs (United States Public Health Service, 1968a).

Some of the factors concerning the problems of introducing a new immunizing product into the population are illustrated in a study of measles vaccine in Erie County, New York, population 1 million (Lennon *et al.*, 1967). A survey was conducted in 1966, 3 years after the licensing of live measles virus vaccine, but prior to the launching of the intensive national effort to eradicate measles. The immunization rates reflected distribution through normal channels of health care. The survey showed that 82% of the vaccinations were received from private physicians, 15% through health department clinics and 3% from other medical facilities, such as hospitals. There was a sharp division of the rate of immunization of susceptible children by socio-economic group. In the urban area, 73% of the upper, 57% of the middle and 19% of the lower, socio-economic group were immunized. As a consequence of these findings, an intensive campaign to immunize the lower socio-economic group in Erie County, concentrated in the core area of the City of Buffalo, was successfully instituted.

Prior to the introduction of vaccine, approximately 400,000 cases had been reported annually in the United States. Because 90–95% of all young adults have a history of clinical measles, it may be assumed that, if all cases were reported, almost 4 million

cases should be reported per year. It may thus be calculated that somewhat over 10% of clinical cases are in fact reported. Since the introduction of measles vaccine and the initiation of more careful surveillance, the percentage of cases reported has undoubtedly improved beyond this figure. In England and Wales, a similar number, over 400,000 cases a year, are reported. On the basis of the same type of calculations, one can estimate that almost half of the cases of clinical measles are therefore reported. In recent years (prior to vaccine introduction) there have been 300–400 measles deaths annually in the United States, and approximately 50–150 deaths annually in England and Wales. Thus, in both countries, the mortality from measles has been approximately two per million population annually.

Measles vaccine became available in Great Britain early in 1966. Clinical trials of live vaccine preceded by inactivated vaccine have indicated that a better degree of long-term protection can be expected from live vaccine alone. The untoward effects observed in the United States in children who had received multiple doses of inactivated vaccine and then, after several years, were given live measles vaccine or were exposed to natural measles have so far not been observed in Great Britain. There is a strong case for the use of live measles virus vaccine alone. The desirability of adopting a vigorous national policy for the elimination of measles was proposed by the Ministry of Health recently (1968a). All susceptible children through the age of 15 years are recommended to receive a dose of live attenuated virus. This should be given during the 2nd year of life and not earlier than 9 months of age.

Other vaccines

Comments concerning the use of certain vaccines which are used only under special circumstances may be of interest. The status of the vaccines in the United States will be compared to that in Great Britain whenever possible.

While exposure to rabies in the United Kingdom would be exceptional, rabies is a problem of significant proportions in the United States. Fig. 9 shows the trends in animal rabies in the United States from 1953 to 1966. Rabies in domestic animals, largely dogs, livestock and cats, has fallen sharply, while there has been an increase in rabies in wildlife, especially skunks, foxes, and bats (United States Public Health Service, 1967). However, the number of human deaths from rabies declined from thirty-four in 1946 to one or two annually in recent years. This decline has resulted in great part from a reduction in rabies in dogs through immunization (United States Public Health Service, 1967). As prophylaxis following proven or assumed exposure, an estimated

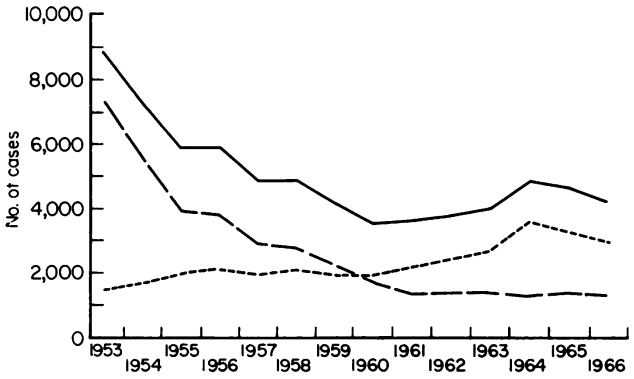

FIG. 9. Reported rabies in domestic and wild animals in the United States, 1953–66 (United States Public Health Service, 1967). —, Total; . . . , wild; — — —, domestic.

30,000 persons received rabies vaccine and approximately 8000 also received anti-rabies serum in 1966.

BCG is recommended for tuberculin-negative children between the ages of 10 and 13 years, at the discretion of the Medical Officer of Health in England and Wales. In certain areas, BCG is given as a routine in infancy. On the other hand, BCG vaccine in the United States is advised only for infants or children who are at special risk of known heavy exposure to tuberculosis in their immediate environment, and, as a consequence, is used far less frequently. In England and Wales in 1966, 640,000 school children and tuberculosis contacts were skin-tested. Five hundred and fourteen thousand were found to be tuberculin-negative, and of these, 479,000 were vaccinated with BCG. Twenty-nine per cent of contacts and 14% of school children were tuberculin-positive. In 1966, there were approximately 15,000 tuberculosis notifications in England and Wales, or a rate of twenty-one per 100,000 population (Ministry of Health, 1967a). In the United States, there were approximately 48,000 new active cases, or a rate of twenty-four per 100,000 (United States Public Health Service, 1968b), with a low of 6·5 in North Dakota and a high of 55·5 in Alaska. At the end of 1966, there were 316,000 cases under supervision because of a tuberculous lesion in England and Wales, and at the same time there were 320,000 persons in the United States on state and local health department tuberculosis registers. There are undoubtedly significant differences in recording and classification contributing to these data, and they may not be directly comparable.

The general recommendations for the use of influenza vaccine are quite similar in the two countries—namely, for the protection of those individuals suffering from certain chronic diseases for whom influenza might prove to be an unusual medical risk. In addition, in the United States, all individuals over the age of 65, a group in which there

is excess mortality accompanying influenza epidemics, are recommended to have annual doses of influenza vaccine. Influenza vaccine is not recommended for general use in the population.

The live attenuated mumps virus vaccine was introduced in the United States early in 1968. Seroconversion and clinical protection have been demonstrated in field trials to last for at least 3 years, which is the longest period of follow-up. The product appears to be free of toxicity. The long-term duration of protection is under continued study. The United States Public Health Service Advisory Committee on Immunization Practices has suggested that live mumps vaccine be considered for use in children approaching puberty, in adolescents, and in adults, especially males, if they have not had mumps (United States Public Health Service, 1967). The vaccine should also be considered in certain institutional settings. The vaccine is not recommended for routine use in younger children, although it is not specifically contraindicated. The rationale for the use of mumps vaccine lies in the prevention of certain complications. Approximately 15% of reported cases of mumps occur after the onset of puberty. Orchitis has been observed in 20% or more of post-pubertal males, and other organ systems, including the central nervous system, are occasionally involved. Sequelae are uncommon, although unilateral deafness is not a rare event (Karzon, 1968a). In terms of public health priorities, the Advisory Committee has stated that mumps immunization programs should not pre-empt other public health programs of established importance. However, should observation confirm the long-term effectiveness and freedom from toxicity, it may be predicted that increasing use will be made of mumps vaccine, both in private practice and public programs.*

A live attenuated rubella virus vaccine is at the present time undergoing field trials in the United States in closed and semi-closed populations. Seroconversion and clinical protection are of a high order. The attenuated virus replicates and can be recovered from the pharynx, although it does not appear to be transmissible. Studies are continuing to evaluate the safety of the rubella vaccine in the open population and in adults, before a licensed product can be made available.

Simultaneous use of immunizing agents

There are several factors which have tended to support the recommendation that administration of immunizing agents be separated whenever feasible, especially when one is a live vaccine. The bases for this recommendation are two-fold: (1) possible interference between two or more antigens, and (2) possible enhancement of toxicity. An example of the latter is the provocation effect of DTP on poliomyelitis infection. It is recommended that administration of two live vaccines be separated by 4 weeks in the United States and 3–4 weeks in England and Wales. The need for separation of antigens is causing an increasing hardship in scheduling visits of small infants. In the United States there are four live products—poliovirus, measles, smallpox and mumps—and a fifth live product, rubella, may soon be available. It is, therefore, of more than theoretical interest to determine the legitimacy of the objections which have been raised to simultaneous use of live vaccines. Studies are in progress to explore the antibody responses to multiple vaccines and possible enhancement of toxicity. If simultaneous administration were feasible, a multivalent product would be of great public health significance. The earliest schedules of vaccination and immunization procedures suggested by the Ministry of Health. In the recommended that DTP and live vaccine other than live poliovaccine be separated by 3–4 weeks. Such a restriction has not been placed in the United States, and at the present time no ill-effects have been documented from simultaneous DTP and live vaccine administration. Recent studies have shown that measles or yellow fever vaccine virus may depress, at least in part, the replication of a second virus, presumably on the basis of interferon production. The maximum inhibition is during the second week following administration of the first virus. Thus, the recommendation that yellow fever vaccine be given at least 4 days before primary vaccination against smallpox (Ministry of Health, 1967a) may be expected to decrease the effectiveness of the smallpox take and antibody response (Karzon, 1968b).

Discussion and conclusions

There has been a consistent downward trend in the morbidity, mortality and residua due to most infectious diseases in the developed countries of the world. Improvements in the social and economic sphere and the control of environmental human waste and arthropod vectors have played major roles. However, the widespread use of immunizing agents has notably contributed to this decline. Society is becoming increasingly expectant that preventable disease will indeed be prevented.

The present review is a modest beginning in the effort to relate official policy on immunization practice in the United States and England and Wales, and the actual use of various biological

* Recently, the Advisory Committee has liberalized its recommendations for mumps vaccine and suggests consideration be given to immunizing all susceptible children over 1 year of age. However, the position was reaffirmed that mumps vaccine programs should not be allowed to take priority over essential ongoing health activities. U.S. Public Health Service (1968d).

products, with trends in morbidity and mortality of preventable diseases. The settings for the study form an interesting backdrop. Child health practices are quite distinct in England and Wales under the tripartite National Health Service Program, when compared to the United States, where a combination of the board-certified pediatrician and the general practitioner, as well as public agencies such as the well-baby clinic and hospital outpatient department, all participate in aspects of preventive and curative medicine. Through the two different pathways, similar mechanisms for evaluation of optimal immunization schedules have evolved. Also, it is increasingly evident that the specific recommendations have drawn more closely together. There have been minor differences of emphasis. For example, the American philosophy has shown less concern with immunodepression due to maternal antibody and immunological immaturity and has regularly immunized beginning at 8–12 weeks, with combined vaccine, adding a third dose to increase efficiency of the program. In Great Britain, there has evidently been a greater concern regarding the risks of smallpox immunization, which has resulted in the lower levels of immunization attained with smallpox vaccine.

The very conspicuous disparities in immunization rates, based upon social class and reflected in low immunization rates in such areas as urban Negro ghettos and rural poverty areas, have posed one of the major residual public health problems in infectious disease control in the United States. It would be of interest to study the acceptance of various immunizing procedures by social class in Great Britain, to determine whether the freely available services may be unevenly distributed in a similar fashion, based on cultural and behavioral characteristics of population groups. For example, poliomyelitis in the United States, once a disease with relative selectivity for the upper classes with a high level of environmental and personal hygiene, is now a disease of the lower classes, in residual pockets with inadequate immunization. It has been generally observed that immunization rates in a given population bear a direct relationship to the general extent of contact between the infant and those charged with early child health care. That is, children who attend a physician only for episodic illness and do not receive regular well-baby care will have poorer immunization records. A corollary of this would state that improvement of the general level of immunization would be tied to an improvement in the general level of infant care and regularity of contact between the family and health facilities. However, the latter situation is not always simple to achieve, and more direct efforts aimed specifically at attaining higher immunization rates can be successful, both as single mass community campaigns or in some form of ongoing program.

It is hoped that the information presented and the interpretations which have resulted will be a stimulus for others to continue to study comparative immunization practices.

Acknowledgments

This paper could not have been written without the help and advice of Dr A. T. Roden of the Ministry of Health, who kindly provided much of the published and unpublished information from England and Wales. For data from the United States, I have leaned heavily on the Reports of the National Communicable Disease Center of the United States Public Health Service. In particular, I would like to thank Dr F. Robert Freckleton and Dr Alexander Langmuir and their staffs for their encouragement and for permission to use data placed at my disposal. For errors in presentation or interpretation, I lay personal claim. Morbidity and mortality information has been obtained from publications of the Ministry of Health, for which I would like to make acknowledgement to the Controller of Her Majesty's Stationery Office.

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EXHIBIT 350

THE VACCINE SCHEDULE 1950-2018

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The first vaccine – Smallpox

- Invented by Edward Jenner in 1796
  - *Jenner noticed that milkmaids who were exposed to cowpox, a milder disease similar to smallpox, did not develop smallpox to the same extent as others*
  - *He developed method of arm-to-arm inoculation: a small amount of pus from one person's blisters was inoculated into the arm of another*

Late 1940's

- In the early part of the century, scientific progress had allowed for the development of new vaccines and large scale vaccine production

- Recommended vaccines:
  - *DTP (Diptheria, Tetanus, Pertussis)*
  - *Smallpox*

Late 1950's

- Oral Polio Vaccine (OPV) – licensed in 1955

- Recommended vaccines:
  - *DTP (Diphtheria, Tetanus, Pertussis)*
  - *Smallpox*
  - *Polio (OPV)*

Late 1960's

- Vaccines against Measles (1963), Mumps (1967) and Rubella (1969) were developed

- Recommended vaccines:
  - *DTP (Diphtheria, Tetanus, Pertussis)*
  - *Smallpox*
  - *Polio (OPV)*
  - *Measles*
  - *Mumps*
  - *Rubella*

1970's

- Smallpox was declared eradicated and use of smallpox vaccine was discontinued in 1972
- Measles, Mumps, Rubella vaccine was combined into MMR

- Recommended vaccines:
  - *DTP (Diphtheria, Tetanus, Pertussis)*
  - *Polio (OPV)*
  - *MMR (Measles, Mumps, Rubella)*

1985-1994

- Hib vaccine developed and added to the schedule

- Recommended vaccines:
  - *DTP (Diphtheria, Tetanus, Pertussis)*
  - *Polio (OPV)*
  - *MMR (Measles, Mumps, Rubella)*
  - *Hib (Haemophilius influenzae Type B)*

1994-1995

- Hepatitis B was added to the schedule as a routine vaccination instead of being used only for high risk groups

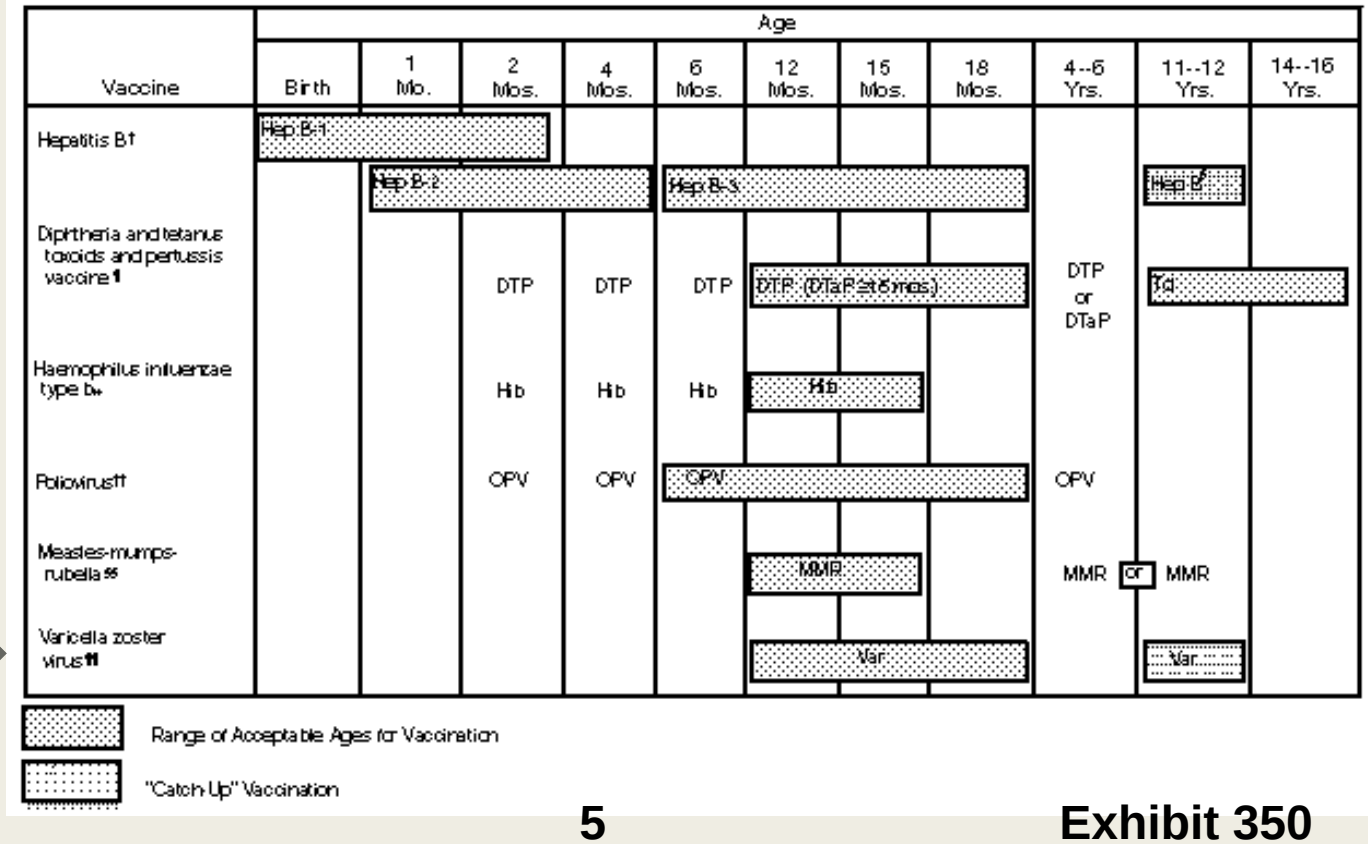
- Recommended vaccines:
  - *DTP (Diphtheria, Tetanus, Pertussis)*
  - *Polio (OPV)*
  - *MMR (Measles, Mumps, Rubella)*
  - *Hib (Haemophilius influenzae Type B)*
  - *Hepatitis B*

1995 Vaccine Schedule

Vaccine	Birth	2 Months	4 Months	6 Months	12 Months	15 Months	18 Months	4–6 Years	11–12 Years	14–16 Years
Hepatitis B	HB-1	HB-2		HB-3						
Diphtheria-Tetanus-Pertussis (DTP)		DTP	DTP	DTP	DTP or DTaP ≥ at 15 months			DTP or DTaP	Td	
Haemophilus influenzae type b		Hib	Hib	Hib	Hib					
Poliovirus		OPV	OPV	OPV				OPV		
Measles-Mumps-Rubella					MMR			MMR or MMR		

1996 Vaccine Schedule

FIGURE 1. Recommended childhood immunization schedule* — United States, July–December 1996



1998 Vaccine Schedule

FIGURE 1. Recommended childhood immunization schedule* — United States, January–December 1999

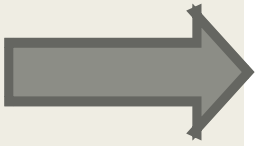
Vaccine	Age: Birth	1 mo	2 mos	4 mos	6 mos	12 mos	15 mos	18 mos	4–6 yrs	11–12 yrs	14–16 yrs
Hepatitis B†	Hep B	Hep B			Hep B					Hep B	
Diphtheria and tetanus toxoids and pertussis§			DTaP	DTaP	DTaP		DTaP		DTaP	Td	
H. influenzae type b¶			Hib	Hib	Hib	Hib					
Poliovirus**			IPV	IPV	Polio				Polio		
Rotavirus††			*Rv*	*Rv*	*Rv*						
Measles-mumps-rubella§§						MMR			MMR	MMR	
Varicella¶¶						Var				Var	

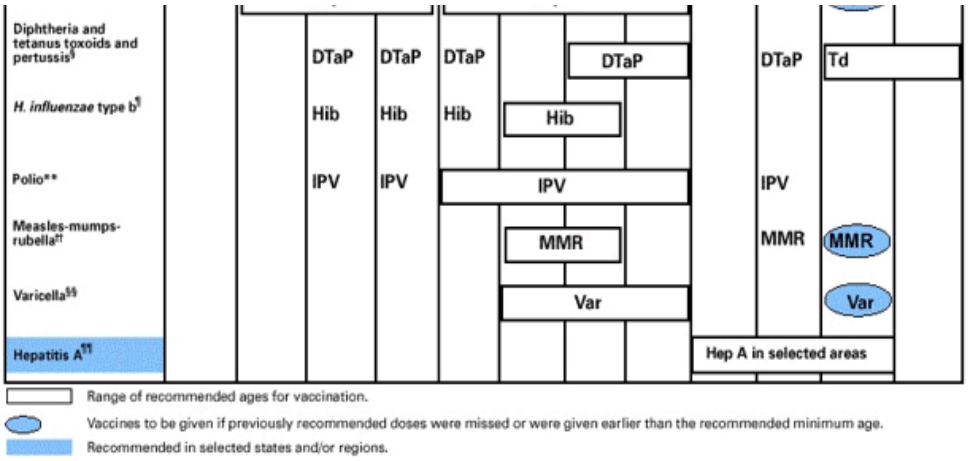
Range of Acceptable Ages for vaccination

Vaccines to be Assessed and Administered if Necessary

Incorporation of this new vaccine into clinical practice may require additional time and resources from health-care providers.

Rotavirus vaccine removed from the schedule





On October 22, 1999, the Advisory Committee on Immunization Practices (ACIP) recommended that Rotashield® (rhesus rotavirus vaccine-tetravalent [RRV-TV]), the only U.S.-licensed rotavirus vaccine, no longer be used in the United States (*MMWR*, Vol. 48, No. 43, November 5, 1999). Parents should be reassured that children who received rotavirus vaccine before July 1999 are not now at increased risk for intussusception.

* This schedule indicates the recommended ages for routine administration of licensed childhood vaccines as of November 1, 1999. Any dose not given at the recommended age should be given as a "catch-up" vaccination at any subsequent visit when indicated and feasible. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' package inserts for detailed recommendations.

† **Infants born to hepatitis B surface antigen (HBsAg)-negative mothers** should receive the first dose of hepatitis B vaccine (Hep B) by age 2 months. The second dose should be administered at least 1 month after the first dose. The third dose should be administered at least 4 months after the first dose and at least 2 months after the second dose, but not before age 6 months. **Infants born to HBsAg-positive mothers** should receive Hep B and 0.5 mL hepatitis B immune globulin (HBIG) within 12 hours of birth at separate sites. The second dose is recommended at age 1–2 months and the third dose at age 6 months. **Infants born to mothers whose HBsAg status is unknown** should receive Hep B within 12 hours of birth. Maternal blood should be drawn at delivery to determine the mother's HBsAg status; if the HBsAg test is positive, the infant should receive HBIG as soon as possible (no later than age 1 week). **All children and adolescents (through age 18 years)** who have not been vaccinated against hepatitis B may begin the series during any visit. Providers should make special efforts to vaccinate children who were born in or whose parents were born in areas of the world where hepatitis B virus infection is moderately or highly endemic.

§ The fourth dose of diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP) can be administered as early as age 12 months, provided 6 months have elapsed since the third dose and the child is unlikely to return at age 15–18 months. Tetanus and diphtheria toxoids (Td) is recommended at age 11–12 years if at least 5 years have elapsed since the last dose of diphtheria and tetanus toxoids and pertussis vaccine (DTP), DTaP, or diphtheria and tetanus toxoids (DT). Subsequent routine Td boosters are recommended every 10 years.

¶ Three *Haemophilus influenzae* type b (Hib) conjugate vaccines are licensed for infant use. If Hib conjugate vaccine (PRP-OMP) (PedvaxHIB® or ComVax® [Merck]) is administered at ages 2 months and 4 months, a dose at age 6 months is not required. Because clinical studies in infants have demonstrated that using some combination products may induce a lower immune response to the Hib vaccine component, DTaP/Hib combination products should not be used for primary vaccination in infants at ages 2, 4, or 6 months unless approved by the Food and Drug Administration for these ages.

** To eliminate the risk for vaccine-associated paralytic poliomyelitis (VAPP), an all-inactivated poliovirus vaccine (IPV) schedule is now recommended for routine childhood polio vaccination in the United States. All children should receive four doses of IPV: at age 2 months, age 4 months, between ages 6 and 18 months, and between ages 4 and 6 years. Oral poliovirus vaccine (OPV) (if available) may be used only for the following special circumstances: 1) mass vaccination campaigns to control outbreaks of paralytic polio; 2) unvaccinated children who will be traveling in <4 weeks to areas where polio is endemic or epidemic; and 3) children of parents who do not accept the recommended number of vaccine injections. Children of parents who do not accept the recommended number of vaccine injections may receive OPV only for the third or fourth dose or both; in this situation, health-care providers should administer OPV only after discussing the risk for VAPP with parents or caregivers. During the transition to an all-IPV schedule, recommendations for the use of remaining OPV supplies in physicians' offices and clinics have been issued by the American Academy of Pediatrics (*Pediatrics*, Vol. 104, No. 6, December 1999).

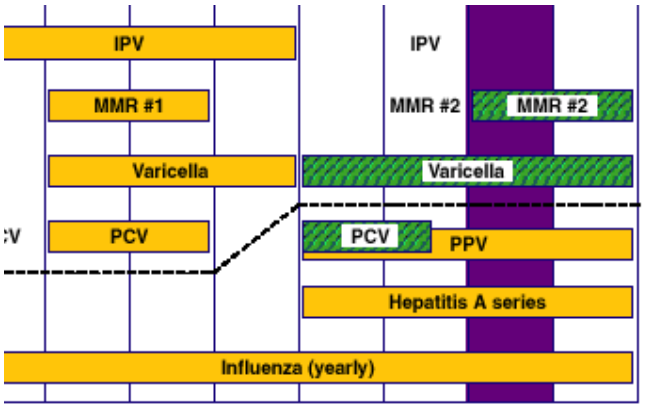
†† The second dose of measles, mumps, and rubella vaccine (MMR) is recommended routinely at age 4–6 years but may be administered during any visit, provided at least 4 weeks have elapsed since receipt of the first dose and that both doses are administered beginning at or after age 12 months. Those who previously have not received the second dose should complete the schedule no later than the routine visit to a health-care provider at age 11–12 years.

§§ Varicella (Var) vaccine is recommended at any visit on or after the first birthday for susceptible children, i.e., those who lack a reliable history of chickenpox (as judged by a health-care provider) and who have not been vaccinated. Susceptible persons aged ≥13 years should receive two doses given at least 4 weeks apart.

¶¶ Hepatitis A vaccine (Hep A) is recommended for use in selected states and regions. Information is available from local public health authorities and *MMWR*, Vol. 48, No. RR-12, October 1, 1999.

Use of trade names and commercial sources is for identification only and does not constitute or imply endorsement by CDC or the U.S. Department of Health and Human Services.

Source: Advisory Committee on Immunization Practices (ACIP), American Academy of Family Physicians (AAFP), and American Academy of Pediatrics (AAP).



ildhood vaccines, as of December 1, 2001, for children through age 18 years. Any dose not ated and feasible. Indicates age groups that warrant special effort to administer those ed during the year. Licensed combination vaccines may be used whenever any components ontraindicated. Providers should consult the manufacturers' package inserts for detailed

vaccine soon after birth and before hospital discharge; the first dose also may be given by vaccine can be used for the birth dose. Monovalent or combination vaccine containing Hep ombination vaccine is used. The second dose should be given at least 4 weeks after the first weeks. The third dose should be given at least 16 weeks after the first dose and at least 8 th dose) should not be administered before age 6 months. Infants born to HBsAg-positive (HBIG) within 12 hours of birth at separate sites. The second dose is recommended at age t age 6 months. Infants born to mothers whose HBsAg status is unknown should receive the hould be drawn at the time of delivery to determine the mother's HBsAg status; if the HBsAg ge 1 week).

ourth dose of DTaP may be administered as early as age 12 months provided that 6 months onths. **Tetanus and diphtheria toxoids (Td)** is recommended at age 11–12 years if at least g vaccine. Subsequent routine Td boosters are recommended every 10 years.
vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB® or ComVax® [Merck]) is /Hib combination products should not be used for primary immunization in infants at age 2,

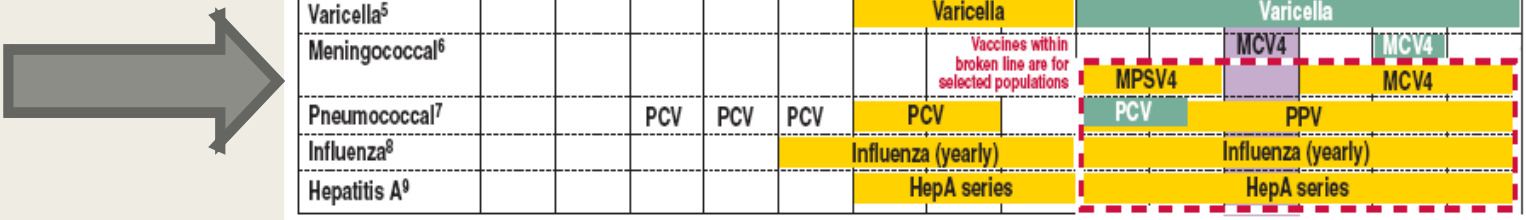
tine childhood poliovirus vaccination in the United States. All children should receive 4 doses

ommended routinely at age 4–6 years but may be administered during any visit provided at ed beginning at or after age 12 months. Those who have not previously received the second

12 months for susceptible children (i.e., those who lack a reliable history of chickenpox). s apart.
CV) is recommended for all children aged 2–23 months and for certain children aged 24–59 tion to PCV for certain high-risk groups. See *MMWR* 2000;49(No. RR-9):1–37.
tes and regions, and for certain high-risk groups. Consult local public health authority and

6 months with certain risk factors (including but not limited to asthma, cardiac disease, sickle dministered to all others wishing to obtain immunity. Children aged ≤12 years should receive years). Children aged ≤8 years who are receiving influenza vaccine for the first time should

nization is available at http://www.cdc.gov/nip or at the National Immunization hotline, 800- d at http://www.cdc.gov/nip/recs/child-schedule.htm. Approved by the **Advisory Committee of Pediatrics** (http://www.aap.org), and the **American Academy of Family Physicians**



This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2005, for children through age 18 years. Any dose not administered at the recommended age should be administered at any subsequent visit, when indicated and feasible. Indicates age groups that warrant special effort to administer those vaccines not previously administered. Additional vaccines might be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and other components of the vaccine are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult respective Advisory Committee on Immunization Practices (ACIP) statements for detailed recommendations. Clinically significant adverse events that follow vaccination should be reported through the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

Range of recommended ages | Catch-up immunization | Assessment at age 11–12 years

1. **Hepatitis B vaccine (HepB).** ***AT BIRTH:*** **All newborns** should receive monovalent HepB soon after birth and before hospital discharge. **Infants born to mothers who are hepatitis B surface antigen (HBsAg)-positive** should receive HepB and 0.5 mL of hepatitis B immune globulin (HBIG) within 12 hours of birth. **Infants born to mothers whose HBsAg status is unknown** should receive HepB within 12 hours of birth. The mother should have blood drawn as soon as possible to determine her HBsAg status; if HBsAg-positive, the infant should receive HBIG as soon as possible (no later than age 1 week). **For infants born to HBsAg-negative mothers,** the birth dose can be delayed in rare circumstances but only if a physician's order to withhold the vaccine and a copy of the mother's original HBsAg-negative laboratory report are documented in the infant's medical record. ***FOLLOWING THE BIRTH DOSE:*** The HepB series should be completed with either monovalent HepB or a combination vaccine containing HepB. The second dose should be administered at age 1–2 months. The final dose should be administered at age ≥24 weeks. Administering four doses of HepB is permissible (e.g., when combination vaccines are administered after the birth dose); however, if monovalent HepB is used, a dose at age 4 months is not needed. **Infants born to HBsAg-positive mothers** should be tested for HBsAg and antibody to HBsAg after completion of the HepB series at age 9–18 months (generally at the next well-child visit after completion of the vaccine series).
2. **Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP).** The fourth dose of DTaP may be administered as early as age 12 months, provided 6 months have elapsed since the third dose and the child is unlikely to return at age 15–18 months. The final dose in the series should be administered at age ≥4 years. **Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap adolescent preparation)** is recommended at age 11–12 years for those who have completed the recommended childhood DTP/DTaP vaccination series and have not received a tetanus and diphtheria toxoids (Td) booster dose. Adolescents aged 13–18 years who missed the age 11–12-year Td/Tdap booster dose should also receive a single dose of Tdap if they have completed the recommended childhood DTP/DTaP vaccination series. **Subsequent Td** boosters are recommended every 10 years.
3. ***Haemophilus influenzae* type b conjugate vaccine (Hib).** Three Hib conjugate vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB® or ComVax® [Merck]) is administered at ages 2 and 4 months, a dose at age 6 months is not required. DTaP/Hib combination products should not be used for primary immunization in infants at ages 2, 4, or 6 months but may be used as boosters after any Hib vaccine. The final dose in the series should be administered at age ≥12 months.
4. **Measles, mumps, and rubella vaccine (MMR).** The second dose of MMR is recommended routinely at age 4–6 years but may be administered during any visit, provided at least 4 weeks have elapsed since the first dose and both doses are administered at or after age 12 months. Children who have not previously received the second dose should complete the schedule by age 11–12 years.
5. **Varicella vaccine.** Varicella vaccine is recommended at any visit at or after age 12 months for susceptible children (i.e., those who lack a reliable history of varicella). Susceptible persons aged ≥13 years should receive 2 doses administered at least 4 weeks apart.
6. **Meningococcal vaccine (MCV4).** Meningococcal conjugate vaccine (MCV4) should be administered to all children at age 11–12 years as well as to unvaccinated adolescents at high school entry (age 15 years). Other adolescents who wish to decrease their risk for meningococcal disease may also be vaccinated. All college freshmen living in dormitories should also be vaccinated, preferably with MCV4, although **meningococcal polysaccharide vaccine (MPSV4)** is an acceptable alternative. Vaccination against invasive meningococcal disease is recommended for children and adolescents aged ≥2 years with terminal complement deficiencies or anatomic or functional asplenia and for certain other high risk groups (see *MMWR* 2005;54[No. RR-7]); use MPSV4 for children aged 2–10 years and MCV4 for older children, although MPSV4 is an acceptable alternative.
7. **Pneumococcal vaccine.** The heptavalent **pneumococcal conjugate vaccine (PCV)** is recommended for all children aged 2–23 months and for certain children aged 24–59 months. The final dose in the series should be administered at age ≥12 months. **Pneumococcal polysaccharide vaccine (PPV)** is recommended in addition to PCV for certain high-risk groups. See *MMWR* 2000;49(No. RR-9).
8. **Influenza vaccine.** Influenza vaccine is recommended annually for children aged ≥6 months with certain risk factors (including, but not limited to, asthma, cardiac disease, sickle cell disease, human immunodeficiency virus infection, diabetes, and conditions that can compromise respiratory function or handling of respiratory secretions or that can increase the risk for aspiration), health-care workers, and other persons (including household members) in close contact with persons in groups at high risk (see *MMWR* 2005;54[No. RR-8]). In addition, healthy children aged 6–23 months and close contacts of healthy children aged 0–5 months are recommended to receive influenza vaccine because children in this age group are at substantially increased risk for influenza-related hospitalizations. For healthy, nonpregnant persons aged 5–49 years, the intranasally administered, live, attenuated influenza vaccine (LAIV) is an acceptable alternative to the intramuscular trivalent inactivated influenza vaccine (TIV). See *MMWR* 2005;54(No. RR-8). Children receiving TIV should be administered an age-appropriate dosage (0.25 mL for children aged 6–35 months or 0.5 mL for children aged ≥3 years). Children aged ≤8 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by at least 4 weeks for TIV and at least 6 weeks for LAIV).
9. **Hepatitis A vaccine (HepA).** HepA is recommended for all children at age 1 year (i.e., 12–23 months). The 2 doses in the series should be administered at least 6 months apart. States, counties, and communities with existing HepA vaccination programs for children aged 2–18 years are encouraged to maintain these programs. In these areas, new efforts focused on routine vaccination of children aged 1 year should enhance, not replace, ongoing programs directed at a broader population of children. HepA is also recommended for certain high risk groups (see *MMWR* 1999;48[No. RR-12]).

The Childhood and Adolescent Immunization Schedule is approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/nip/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.afp.org).

2007 Vacine Schedule

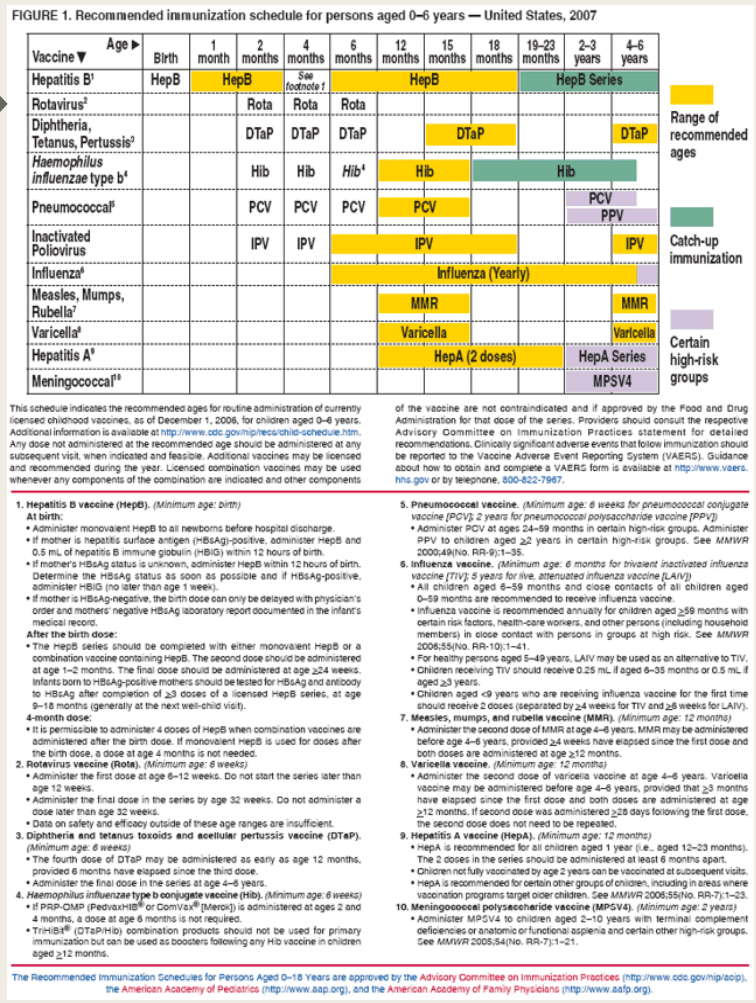


FIGURE 1. Recommended immunization schedule for persons aged 0–6 years — United States, 2007

Vaccine ▼ / Age ▶	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB		*See footnote 1*	HepB				HepB Series		
Rotavirus[2]			Rota	Rota	Rota						
Diphtheria, Tetanus, Pertussis[3]			DTaP	DTaP	DTaP		DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	*Hib*[4]	Hib		Hib			
Pneumococcal[5]			PCV	PCV	PCV	PCV				PCV	PPV
Inactivated Poliovirus			IPV	IPV	IPV						IPV
Influenza[6]					Influenza (Yearly)						
Measles, Mumps, Rubella[7]						MMR					MMR
Varicella[8]						Varicella					Varicella
Hepatitis A[9]						HepA (2 doses)				HepA Series	
Meningococcal[10]										MPSV4	

Range of recommended ages

Catch-up immunization

Certain high-risk groups

This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2006, for children aged 0–6 years. Additional information is available at http://www.cdc.gov/nip/recs/child-schedule.htm. Any dose not administered at the recommended age should be administered at any subsequent visit, when indicated and feasible. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and other components of the vaccine are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult the respective Advisory Committee on Immunization Practices statement for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

1. **Hepatitis B vaccine (HepB).** *(Minimum age: birth)*
   At birth:
   - Administer monovalent HepB to all newborns before hospital discharge.
   - If mother is hepatitis surface antigen (HBsAg)-positive, administer HepB and 0.5 mL of hepatitis B immune globulin (HBIG) within 12 hours of birth.
   - If mother's HBsAg status is unknown, administer HepB within 12 hours of birth. Determine the HBsAg status as soon as possible and if HBsAg-positive, administer HBIG (no later than age 1 week).
   - If mother is HBsAg-negative, the birth dose can only be delayed with physician's order and mothers' negative HBsAg laboratory report documented in the infant's medical record.

   After the birth dose:
   - The HepB series should be completed with either monovalent HepB or a combination vaccine containing HepB. The second dose should be administered at age 1–2 months. The final dose should be administered at age ≥24 weeks. Infants born to HBsAg-positive mothers should be tested for HBsAg and antibody to HBsAg after completion of ≥3 doses of a licensed HepB series, at age 9–18 months (generally at the next well-child visit).

   4-month dose:
   - It is permissible to administer 4 doses of HepB when combination vaccines are administered after the birth dose. If monovalent HepB is used for doses after the birth dose, a dose at age 4 months is not needed.
2. **Rotavirus vaccine (Rota).** *(Minimum age: 6 weeks)*
   - Administer the first dose at age 6–12 weeks. Do not start the series later than age 12 weeks.
   - Administer the final dose in the series by age 32 weeks. Do not administer a dose later than age 32 weeks.
   - Data on safety and efficacy outside of these age ranges are insufficient.
3. **Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP).** *(Minimum age: 6 weeks)*
   - The fourth dose of DTaP may be administered as early as age 12 months, provided 6 months have elapsed since the third dose.
   - Administer the final dose in the series at age 4–6 years.
4. ***Haemophilus influenzae* type b conjugate vaccine (Hib).** *(Minimum age: 6 weeks)*
   - If PRP-OMP (PedvaxHIB® or ComVax® [Merck]) is administered at ages 2 and 4 months, a dose at age 6 months is not required.
   - TriHiBit® (DTaP/Hib) combination products should not be used for primary immunization but can be used as boosters following any Hib vaccine in children aged ≥12 months.
5. **Pneumococcal vaccine.** *(Minimum age: 6 weeks for pneumococcal conjugate vaccine [PCV]; 2 years for pneumococcal polysaccharide vaccine [PPV])*
   - Administer PCV at ages 24–59 months in certain high-risk groups. Administer PPV to children aged ≥2 years in certain high-risk groups. See *MMWR* 2000;49(No. RR-9):1–35.
6. **Influenza vaccine.** *(Minimum age: 6 months for trivalent inactivated influenza vaccine [TIV]; 5 years for live, attenuated influenza vaccine [LAIV])*
   - All children aged 6–59 months and close contacts of all children aged 0–59 months are recommended to receive influenza vaccine.
   - Influenza vaccine is recommended annually for children aged ≥59 months with certain risk factors, health-care workers, and other persons (including household members) in close contact with persons in groups at high risk. See *MMWR* 2006;55(No. RR-10):1–41.
   - For healthy persons aged 5–49 years, LAIV may be used as an alternative to TIV.
   - Children receiving TIV should receive 0.25 mL if aged 6–35 months or 0.5 mL if aged ≥3 years.
   - Children aged <9 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by ≥4 weeks for TIV and ≥6 weeks for LAIV).
7. **Measles, mumps, and rubella vaccine (MMR).** *(Minimum age: 12 months)*
   - Administer the second dose of MMR at age 4–6 years. MMR may be administered before age 4–6 years, provided ≥4 weeks have elapsed since the first dose and both doses are administered at age ≥12 months.
8. **Varicella vaccine.** *(Minimum age: 12 months)*
   - Administer the second dose of varicella vaccine at age 4–6 years. Varicella vaccine may be administered before age 4–6 years, provided that ≥3 months have elapsed since the first dose and both doses are administered at age ≥12 months. If second dose was administered ≥28 days following the first dose, the second dose does not need to be repeated.
9. **Hepatitis A vaccine (HepA).** *(Minimum age: 12 months)*
   - HepA is recommended for all children aged 1 year (i.e., aged 12–23 months). The 2 doses in the series should be administered at least 6 months apart.
   - Children not fully vaccinated by age 2 years can be vaccinated at subsequent visits.
   - HepA is recommended for certain other groups of children, including in areas where vaccination programs target older children. See *MMWR* 2006;55(No. RR-7):1–23.
10. **Meningococcal polysaccharide vaccine (MPSV4).** *(Minimum age: 2 years)*
    - Administer MPSV4 to children aged 2–10 years with terminal complement deficiencies or anatomic or functional asplenia and certain other high-risk groups. See *MMWR* 2005;54(No. RR-7):1–21.

The Recommended Immunization Schedules for Persons Aged 0–18 Years are approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/nip/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.aafp.org).

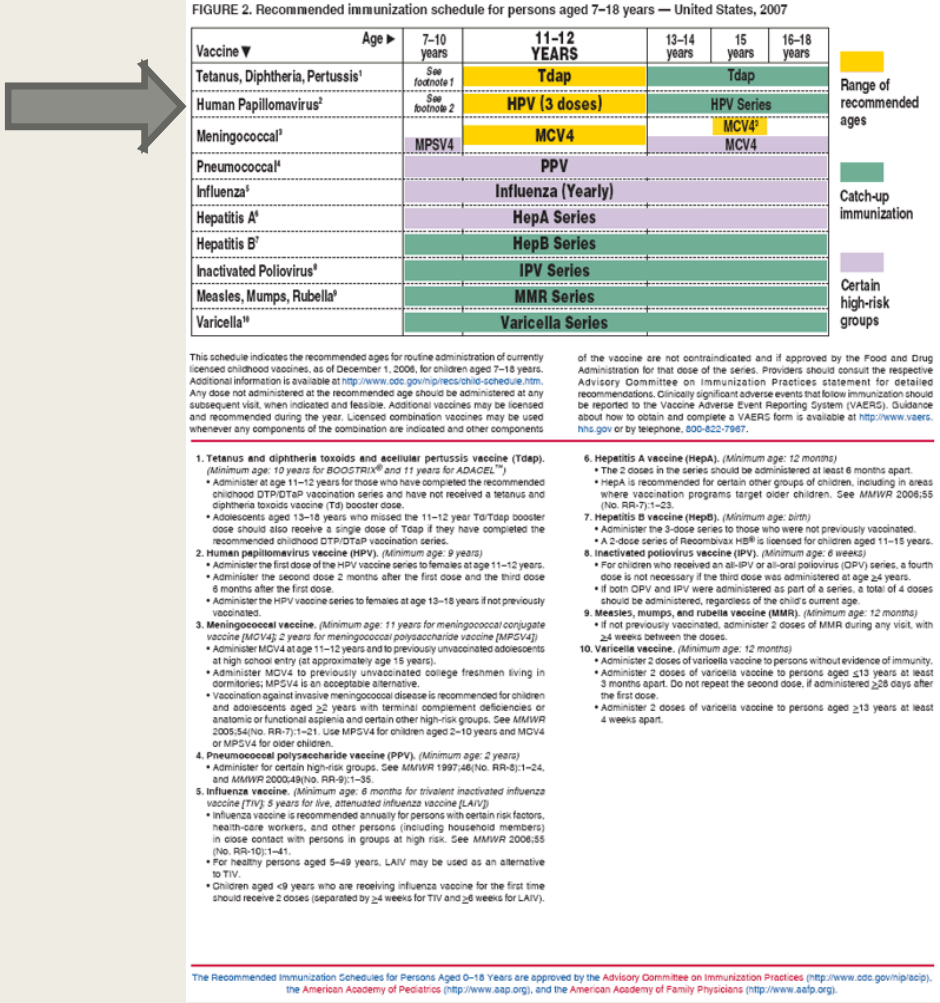


FIGURE 2. Recommended immunization schedule for persons aged 7–18 years — United States, 2007

Vaccine ▼ / Age ▶	7–10 years	11–12 YEARS	13–14 years	15 years	16–18 years
Tetanus, Diphtheria, Pertussis[1]	*See footnote 1*	Tdap	Tdap		
Human Papillomavirus[2]	*See footnote 2*	HPV (3 doses)	HPV Series		
Meningococcal[3]	MPSV4	MCV4	MCV4	MCV4[3]	
Pneumococcal[4]	PPV				
Influenza[5]	Influenza (Yearly)				
Hepatitis A[6]	HepA Series				
Hepatitis B[7]	HepB Series				
Inactivated Poliovirus[8]	IPV Series				
Measles, Mumps, Rubella[9]	MMR Series				
Varicella[10]	Varicella Series				

Range of recommended ages

Catch-up immunization

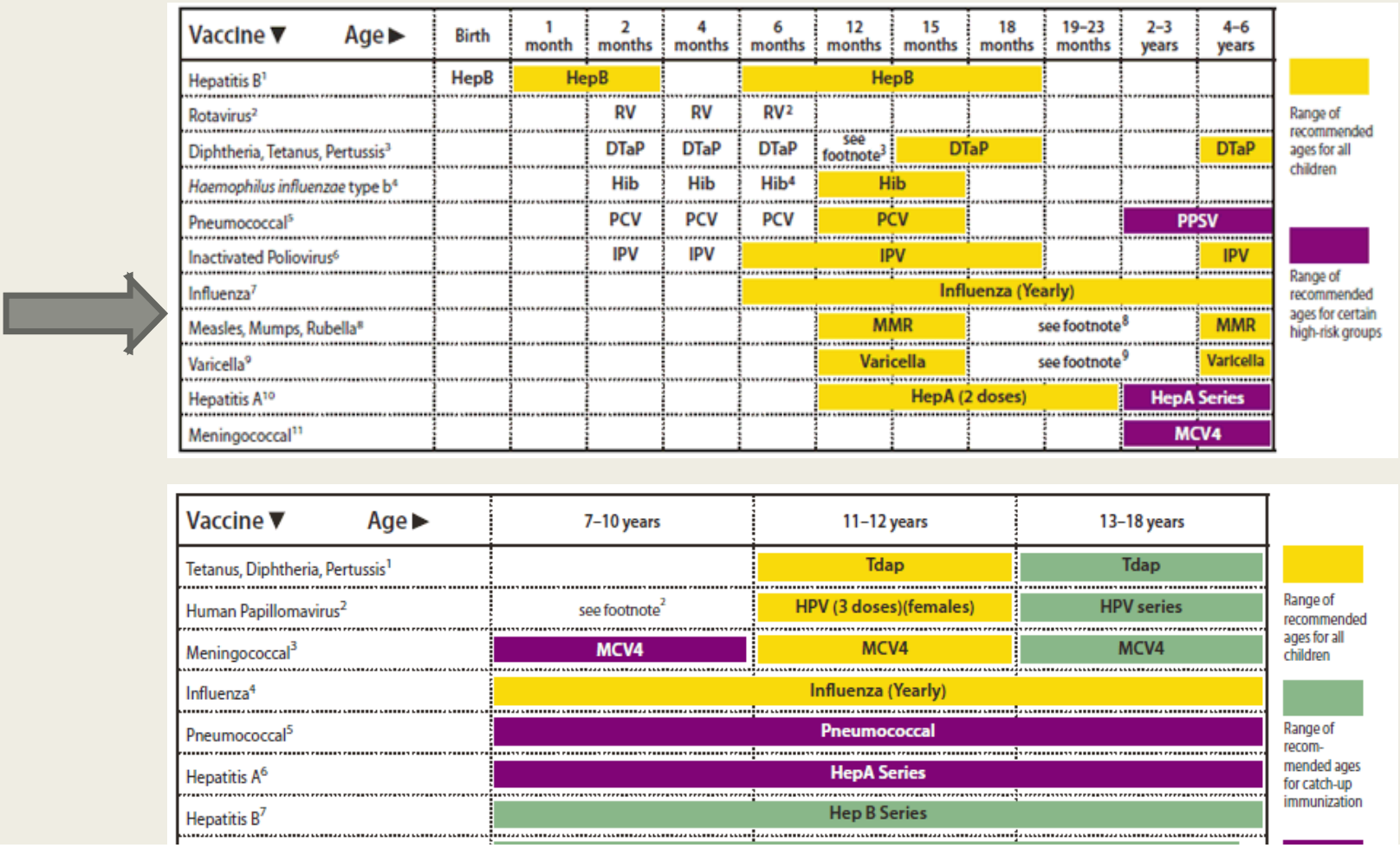
Certain high-risk groups

This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2006, for children aged 7–18 years. Additional information is available at http://www.cdc.gov/nip/recs/child-schedule.htm. Any dose not administered at the recommended age should be administered at any subsequent visit, when indicated and feasible. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and other components of the vaccine are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult the respective Advisory Committee on Immunization Practices statement for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

1. **Tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap).** *(Minimum age: 10 years for BOOSTRIX® and 11 years for ADACEL™)*
   - Administer at age 11–12 years for those who have completed the recommended childhood DTP/DTaP vaccination series and have not received a tetanus and diphtheria toxoids vaccine (Td) booster dose.
   - Adolescents aged 13–18 years who missed the 11–12 year Td/Tdap booster dose should also receive a single dose of Tdap if they have completed the recommended childhood DTP/DTaP vaccination series.
2. **Human papillomavirus vaccine (HPV).** *(Minimum age: 9 years)*
   - Administer the first dose of the HPV vaccine series to females at age 11–12 years.
   - Administer the second dose 2 months after the first dose and the third dose 6 months after the first dose.
   - Administer the HPV vaccine series to females at age 13–18 years if not previously vaccinated.
3. **Meningococcal vaccine.** *(Minimum age: 11 years for meningococcal conjugate vaccine [MCV4]; 2 years for meningococcal polysaccharide vaccine [MPSV4])*
   - Administer MCV4 at age 11–12 years and to previously unvaccinated adolescents at high school entry (at approximately age 15 years).
   - Administer MCV4 to previously unvaccinated college freshmen living in dormitories; MPSV4 is an acceptable alternative.
   - Vaccination against invasive meningococcal disease is recommended for children and adolescents aged ≥2 years with terminal complement deficiencies or anatomic or functional asplenia and certain other high-risk groups. See *MMWR* 2005;54(No. RR-7):1–21. Use MPSV4 for children aged 2–10 years and MCV4 or MPSV4 for older children.
4. **Pneumococcal polysaccharide vaccine (PPV).** *(Minimum age: 2 years)*
   - Administer for certain high-risk groups. See *MMWR* 1997;46(No. RR-8):1–24, and *MMWR* 2000;49(No. RR-9):1–35.
5. **Influenza vaccine.** *(Minimum age: 6 months for trivalent inactivated influenza vaccine [TIV]; 5 years for live, attenuated influenza vaccine [LAIV])*
   - Influenza vaccine is recommended annually for persons with certain risk factors, health-care workers, and other persons (including household members) in close contact with persons in groups at high risk. See *MMWR* 2006;55 (No. RR-10):1–41.
   - For healthy persons aged 5–49 years, LAIV may be used as an alternative to TIV.
   - Children aged <9 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by ≥4 weeks for TIV and ≥6 weeks for LAIV).
6. **Hepatitis A vaccine (HepA).** *(Minimum age: 12 months)*
   - The 2 doses in the series should be administered at least 6 months apart.
   - HepA is recommended for certain other groups of children, including in areas where vaccination programs target older children. See *MMWR* 2006;55 (No. RR-7):1–23.
7. **Hepatitis B vaccine (HepB).** *(Minimum age: birth)*
   - Administer the 3-dose series to those who were not previously vaccinated.
   - A 2-dose series of Recombivax HB® is licensed for children aged 11–15 years.
8. **Inactivated poliovirus vaccine (IPV).** *(Minimum age: 6 weeks)*
   - For children who received an all-IPV or all-oral poliovirus (OPV) series, a fourth dose is not necessary if the third dose was administered at age ≥4 years.
   - If both OPV and IPV were administered as part of a series, a total of 4 doses should be administered, regardless of the child's current age.
9. **Measles, mumps, and rubella vaccine (MMR).** *(Minimum age: 12 months)*
   - If not previously vaccinated, administer 2 doses of MMR during any visit, with ≥4 weeks between the doses.
10. **Varicella vaccine.** *(Minimum age: 12 months)*
    - Administer 2 doses of varicella vaccine to persons without evidence of immunity.
    - Administer 2 doses of varicella vaccine to persons aged ≤13 years at least 3 months apart. Do not repeat the second dose, if administered ≥28 days after the first dose.
    - Administer 2 doses of varicella vaccine to persons aged ≥13 years at least 4 weeks apart.

The Recommended Immunization Schedules for Persons Aged 0–18 Years are approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/nip/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.aafp.org).

2011 Vaccination Schedule



Vaccine ▼ Age ►	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB			HepB						
Rotavirus[2]			RV	RV	RV[2]						
Diphtheria, Tetanus, Pertussis[3]			DTaP	DTaP	DTaP	see footnote[3]	DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	Hib[4]	Hib					
Pneumococcal[5]			PCV	PCV	PCV	PCV				PPSV	
Inactivated Poliovirus[6]			IPV	IPV	IPV						IPV
Influenza[7]					Influenza (Yearly)						
Measles, Mumps, Rubella[8]						MMR	see footnote[8]				MMR
Varicella[9]						Varicella	see footnote[9]				Varicella
Hepatitis A[10]						HepA (2 doses)				HepA Series	
Meningococcal[11]										MCV4	

Range of recommended ages for all children

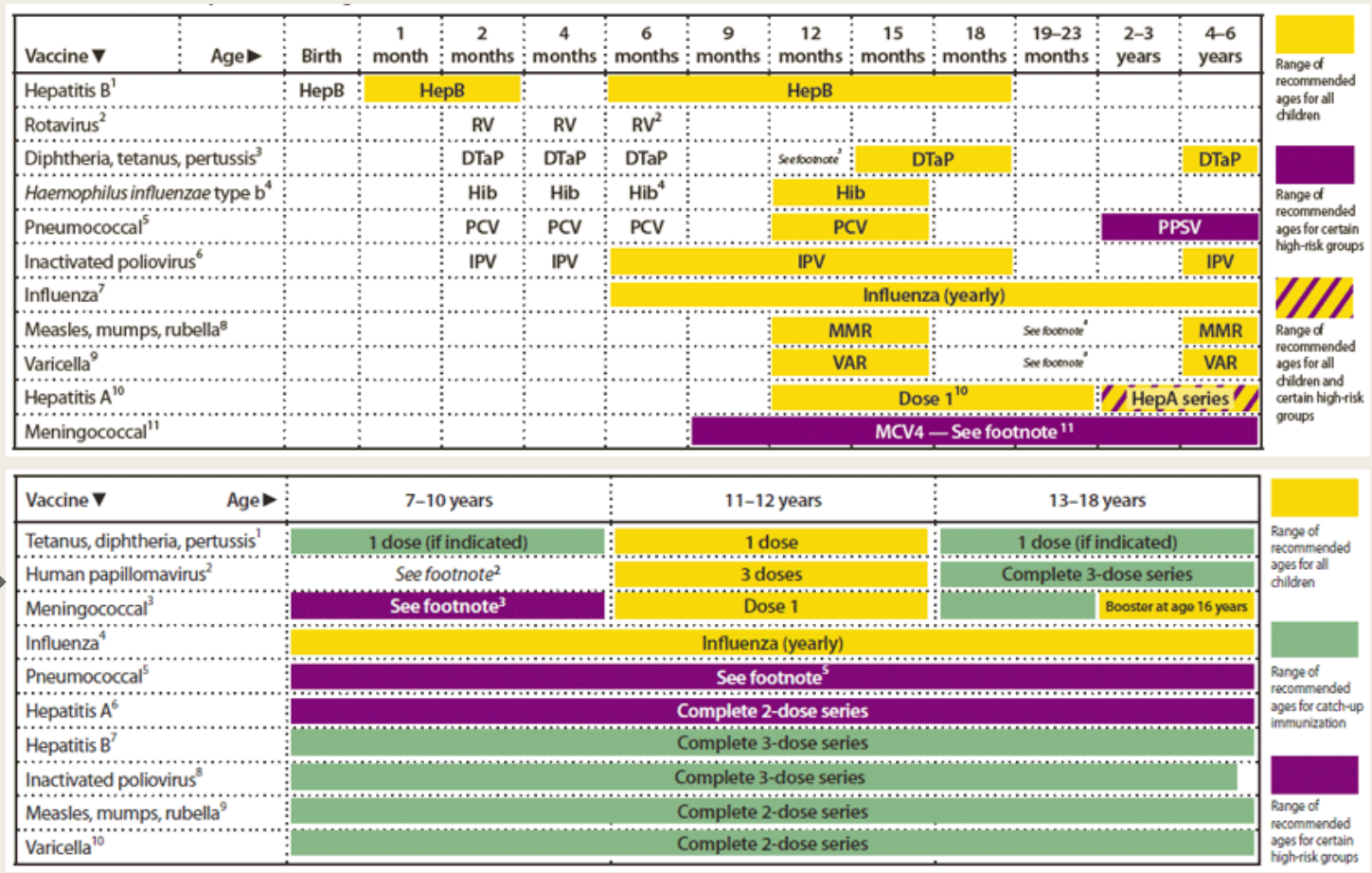
Range of recommended ages for certain high-risk groups

Vaccine ▼ Age ►	7–10 years	11–12 years	13–18 years
Tetanus, Diphtheria, Pertussis[1]		Tdap	Tdap
Human Papillomavirus[2]	see footnote[2]	HPV (3 doses)(females)	HPV series
Meningococcal[3]	MCV4	MCV4	MCV4
Influenza[4]	Influenza (Yearly)		
Pneumococcal[5]	Pneumococcal		
Hepatitis A[6]	HepA Series		
Hepatitis B[7]	Hep B Series		

Range of recommended ages for all children

Range of recommended ages for catch-up immunization

2012 Vaccine Schedule



Vaccine ▼ / Age ▶	Birth	1 month	2 months	4 months	6 months	9 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB			HepB							
Rotavirus[2]			RV	RV	RV[2]							
Diphtheria, tetanus, pertussis[3]			DTaP	DTaP	DTaP		See footnote[3]	DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	Hib[4]		Hib					
Pneumococcal[5]			PCV	PCV	PCV		PCV				PPSV	
Inactivated poliovirus[6]			IPV	IPV	IPV							IPV
Influenza[7]					Influenza (yearly)							
Measles, mumps, rubella[8]							MMR		See footnote[4]			MMR
Varicella[9]							VAR		See footnote[5]			VAR
Hepatitis A[10]							Dose 1[10]				HepA series	
Meningococcal[11]						MCV4 — See footnote[11]						

Range of recommended ages for all children

Range of recommended ages for certain high-risk groups

Range of recommended ages for all children and certain high-risk groups

Vaccine ▼ / Age ▶	7–10 years	11–12 years	13–18 years
Tetanus, diphtheria, pertussis[1]	1 dose (if indicated)	1 dose	1 dose (if indicated)
Human papillomavirus[2]	*See footnote*[2]	3 doses	Complete 3-dose series
Meningococcal[3]	See footnote[3]	Dose 1	Booster at age 16 years
Influenza[4]	Influenza (yearly)		
Pneumococcal[5]	See footnote[5]		
Hepatitis A[6]	Complete 2-dose series		
Hepatitis B[7]	Complete 3-dose series		
Inactivated poliovirus[8]	Complete 3-dose series		
Measles, mumps, rubella[9]	Complete 2-dose series		
Varicella[10]	Complete 2-dose series		

Range of recommended ages for all children

Range of recommended ages for catch-up immunization

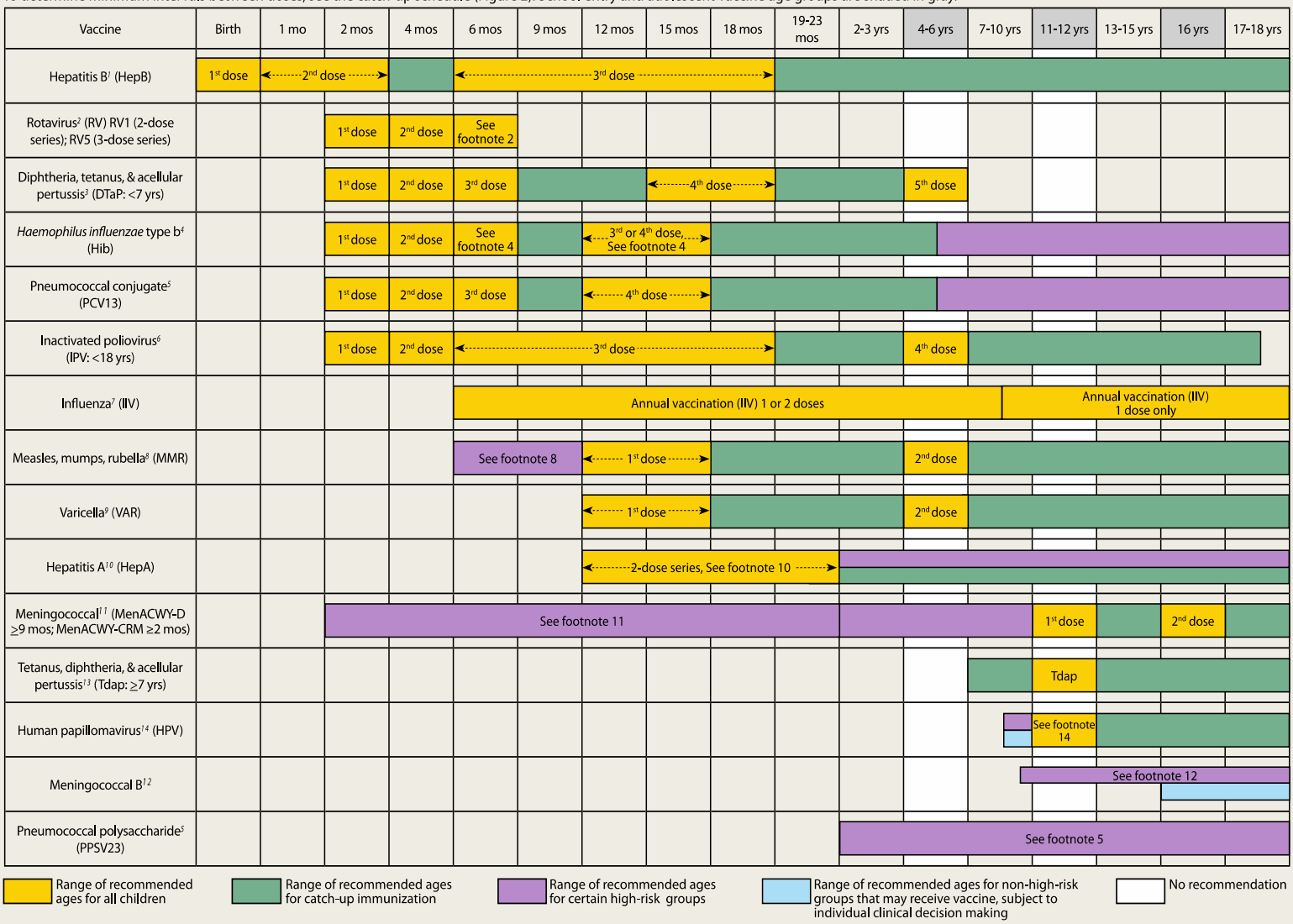
Range of recommended ages for certain high-risk groups

2018 Vaccine Schedule

Figure 1. Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger—United States, 2018.

(FOR THOSE WHO FALL BEHIND OR START LATE, SEE THE CATCH-UP SCHEDULE [FIGURE 2]).

These recommendations must be read with the footnotes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars in Figure 1. To determine minimum intervals between doses, see the catch-up schedule (Figure 2). School entry and adolescent vaccine age groups are shaded in gray.



NOTE: The above recommendations must be read along with the footnotes of this schedule.

EXHIBIT 351

University of Colorado
Anschutz Medical Campus

Vaccination Schedules Past, Present and Future

Is there some rationale?

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COLORADO SCHOOL OF PUBLIC HEALTH

Children's Hospital Colorado

Exhibit 351

Conflict of Interest and disclosures

- **Research and consulting support**
  - DSMB participation for Takeda, Novartis and PATH
  - Advisory Board for J&J and LSHTM
  - Vaccine studies on polio sponsored by Bill and Melinda Gates Foundation

- **Other membership biases**
  - Previous WHO advisor on global vaccine safety
  - Latin American Society for Infectious Diseases
  - Board member of CCIC, Colorado

Objectives

- History of schedules and how designed
- What is the evidence behind current schedules
- New vaccines, new challenges
- Future of vaccine schedules: creative policies in context of need

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[Fom The Merck Manual, Eighth Edition, published 1950]

ROUTINE IMMUNIZATION PROCEDURES

Optional pediatric immunization schedules and timetables for the administration of booster or re-immunization doses are presented. A table outlining the use of human serum immune (gamma) globulin also is included. Although many pertinent details are given, actual dosage must be regulated according to individual circumstances and to the instructions accompanying packages of the various immunizing agents. (For special immunization procedures against such diseases as typhoid fever, yellow fever, cholera, plague, and other conditions not ordinarily included in pediatric practice, *see* the respective chapters.)

BASIC IMMUNIZATION

OPTIONAL SCHEDULE NO. 1

Age	Agent
3 months	Pertussis Vaccine (Alum Precipitated)
4 "	" " " "
5 "	" " " "
6 "	Diphtheria-Tetanus Toxoid (Alum Precipitated)
6 "	Smallpox Vaccine
7 "	Diphtheria-Tetanus Toxoid (Alum Precipitated)
11 "	Schick Test
11 "	Pertussis Vaccine (Alum Precipitated)

OPTIONAL SCHEDULE NO. 2

Age	Agent
As soon as umbilicus is healed and baby is thriving	Smallpox Vaccine
3 months	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
4 "	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
5 "	Pertussis Vaccine (Alum Precipitated)
7 "	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
11 "	Schick Test

BOOSTER DOSES AND RE-IMMUNIZATION

(This schedule applies only when basic immunization has been previously accomplished.)

Age and Indication	Agent
2 years	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
5 "	Diphtheria-Tetanus-Pertussis (Alum Precipitated or Aluminum Hydroxide Adsorbed)
5 "	Schick Test
Every 2 years	Smallpox Vaccine
Every 5 years or upon exposure to smallpox, or during threatened smallpox epidemic	Tetanus Toxoid (Alum Precipitated)
Any age, upon possible exposure to tetanus	Smallpox Vaccine
Any age, upon exposure to diphtheria	Fluid Tetanus Toxoid
Any age, upon exposure to pertussis	Fluid Diphtheria Toxoid
	Pertussis Vaccine (N.B., in Isotonic Saline)

Immunization Schedules in the United States and Great Britain -1967-68

TABLE 1. Recommended schedules for routine immunization

United States*					England and Wales†					
Age	DTP	OPV	M	SP	Age	DTP	OPV	M	SP	BCG
2–3 months	X	X			3–6 months	X	X			
3–4 months	X									
4–5 months	X	X			5–8 months	X	X			
12–18 months	X	X	X		9–14 months	X	X			
12–24 months				X	12–24 months			X	X	
School entry (3–6 years)	X	X		X	School entry (3–6 years)	Td	X		X	
					10–13 years					X
Every 10 years	Td			X‡	School leaving	Td	X		X	

DTP, Diphtheria–tetanus–pertussis vaccine; OPV, oral poliovaccine; M, measles vaccine; SP, smallpox vaccine; Td, tetanus-diphtheria toxoid, adult type.

* Adopted from United States Public Health Service (1967): *Immunization Against Disease* 1966–67 (National Communicable Disease Center publication).

† Adopted from Ministry of Health (1968a,b).

‡ For high risk groups, i.e. health personnel and overseas travel—every 3 years.

Karzon, DT. *Postgrad Med J* 45; 147: 1969

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United States Immunization Schedule 2001

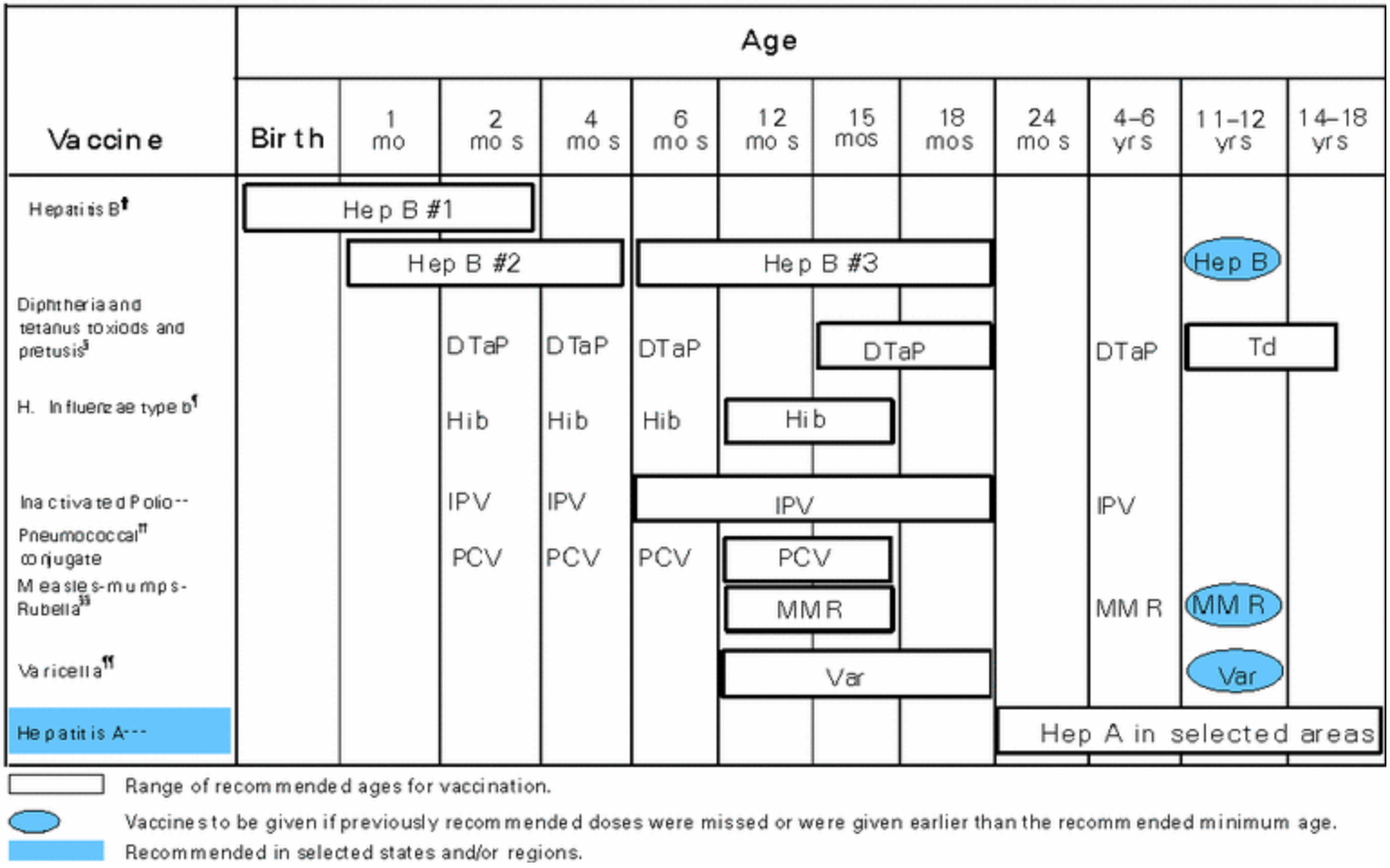

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Exhibit 351

US Child Immunization Schedule 2014

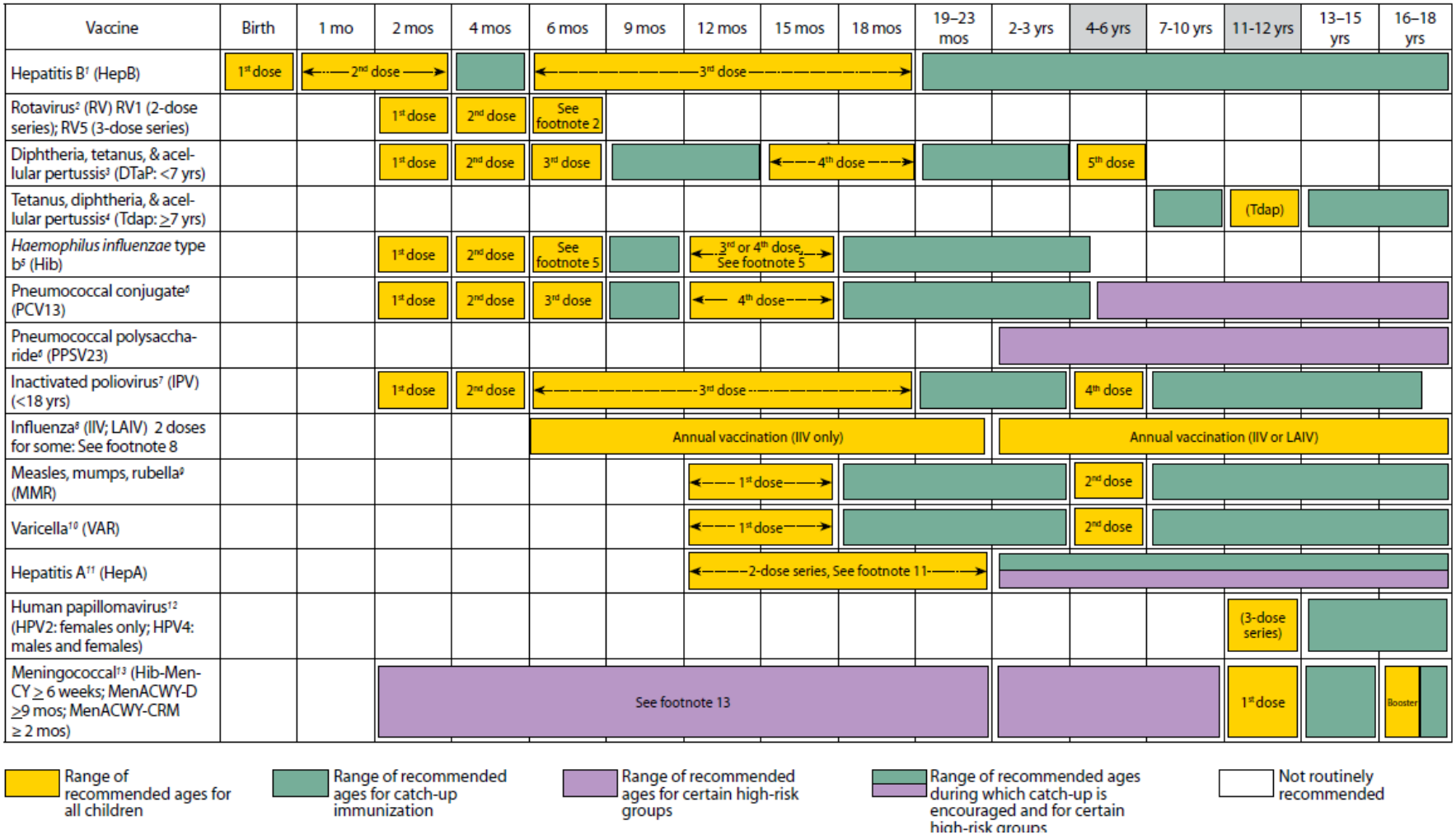

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Exhibit 351

US recommended Catch-Up schedule 2014

Persons aged 4 months through 6 years					
Vaccine	Minimum Age for Dose 1	Minimum Interval Between Doses			
		Dose 1 to dose 2	Dose 2 to dose 3	Dose 3 to dose 4	Dose 4 to dose 5
Hepatitis B[1]	Birth	4 weeks	8 weeks and at least 16 weeks after first dose; minimum age for the final dose is 24 weeks		
Rotavirus[2]	6 weeks	4 weeks	4 weeks[2]		
Diphtheria, tetanus, & acellular pertussis[3]	6 weeks	4 weeks	4 weeks	6 months	6 months[3]
Haemophilus influenzae type b[5]	6 weeks	4 weeks if first dose administered at younger than age 12 months 8 weeks (as final dose) if first dose administered at age 12 through 14 months No further doses needed if first dose administered at age 15 months or older	4 weeks[5] if current age is younger than 12 months and first dose administered at < 7 months old 8 weeks and age 12 months through 59 months (as final dose)[5] if current age is younger than 12 months and first dose administered between 7 through 11 months (regardless of Hib vaccine [PRP-T or PRP-OMP] used for first dose); OR if current age is 12 through 59 months and first dose administered at younger than age 12 months; OR first 2 doses were PRP-OMP and administered at younger than 12 months. No further doses needed if previous dose administered at age 15 months or older	8 weeks (as final dose) This dose only necessary for children aged 12 through 59 months who received 3 (PRP-T) doses before age 12 months and started the primary series before age 7 months	
Pneumococcal[6]	6 weeks	4 weeks if first dose administered at younger than age 12 months 8 weeks (as final dose for healthy children) if first dose administered at age 12 months or older No further doses needed for healthy children if first dose administered at age 24 months or older	4 weeks if current age is younger than 12 months 8 weeks (as final dose for healthy children) if current age is 12 months or older No further doses needed for healthy children if previous dose administered at age 24 months or older	8 weeks (as final dose) This dose only necessary for children aged 12 through 59 months who received 3 doses before age 12 months or for children at high risk who received 3 doses at any age	
Inactivated poliovirus[7]	6 weeks	4 weeks[7]	4 weeks[7]	6 months[7] minimum age 4 years for final dose	
Meningococcal[13]	6 weeks	8 weeks[13]	See footnote 13	See footnote 13	
Measles, mumps, rubella[9]	12 months	4 weeks			
Varicella[10]	12 months	3 months			
Hepatitis A[11]	12 months	6 months			
Persons aged 7 through 18 years					
Tetanus, diphtheria; tetanus, diphtheria, & acellular pertussis[4]	7 years[4]	4 weeks	4 weeks if first dose of DTaP/DT administered at younger than age 12 months 6 months if first dose of DTaP/DT administered at age 12 months or older and then no further doses needed for catch-up	6 months if first dose of DTaP/DT administered at younger than age 12 months	
Human papillomavirus[12]	9 years	Routine dosing intervals are recommended[12]			
Hepatitis A[11]	12 months	6 months			
Hepatitis B[1]	Birth	4 weeks	8 weeks (and at least 16 weeks after first dose)		
Inactivated poliovirus[7]	6 weeks	4 weeks	4 weeks[7]	6 months[7]	
Meningococcal[13]	6 weeks	8 weeks[13]			
Measles, mumps, rubella[9]	12 months	4 weeks			
Varicella[10]	12 months	3 months if person is younger than age 13 years 4 weeks if person is aged 13 years or older			

EXHIBIT 352

Vaccine Excipient Summary

Excipients Included in U.S. Vaccines, by Vaccine

In addition to weakened or killed disease antigens (viruses or bacteria), vaccines contain very small amounts of other ingredients – excipients.

Some excipients are added to a vaccine for a specific purpose. These include:
Preservatives, to prevent contamination. For example, thimerosal.
Adjuvants, to help stimulate a stronger immune response. For example, aluminum salts.
Stabilizers, to keep the vaccine potent during transportation and storage. For example, sugars or gelatin.

Others are residual trace amounts of materials that were used during the manufacturing process and removed. These can include:
Cell culture materials, used to grow the vaccine antigens. For example, egg protein, various culture media.
Inactivating ingredients, used to kill viruses or inactivate toxins. For example, formaldehyde.
Antibiotics, used to prevent contamination by bacteria. For example, neomycin.

The following table lists substances, other than active ingredients (i.e., antigens), shown in the manufacturers' package insert (PI) as being contained in the final formulation of each vaccine. **Note: Substances used in the manufacture of a vaccine but not listed as contained in the final product (e.g., culture media) can be found in each PI, but are not shown on this table.** Each PI, which can be found on the FDA's website (see below) contains a description of that vaccine's manufacturing process, including the amount and purpose of each substance. In most PIs, this information is found in Section 11: "Description."

All information was extracted from manufacturers' package inserts.
The date shown in the Date column of the table is the edition date of the PI is use in February 2020.
If a date contains an asterisk (*), the PI was not dated and this is the date the PI was reviewed for this table.
If in doubt about whether a PI has been updated since this table was prepared, check the FDA's website at:
http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm
All influenza vaccine in this table are 2019-20 northern hemisphere formulation.

Vaccine	Date	Contains
Adenovirus	10/2019	monosodium glutamate, sucrose, D-mannose, D-fructose, dextrose, human serum albumin, potassium phosphate, plasdone C, anhydrous lactose, microcrystalline cellulose, polacrilin potassium, magnesium stearate, cellulose acetate phthalate, alcohol, acetone, castor oil, FD&C Yellow #6 aluminum lake dye
Anthrax (Biothrax)	11/2015	aluminum hydroxide, sodium chloride, benzethonium chloride, formaldehyde
BCG (Tice)	2/2009	glycerin, asparagine, citric acid, potassium phosphate, magnesium sulfate, iron ammonium citrate, lactose
Cholera (Vaxchora)	6/2016	ascorbic acid, hydrolyzed casein, sodium chloride, sucrose, dried lactose, sodium bicarbonate, sodium carbonate
Dengue (Dengvaxia)	6/2019	sodium chloride, essential amino acids (including L-phenylalanine), non-essential amino acids, L-arginine hydrochloride, sucrose, D-trehalose dihydrate, D-sorbitol, trometamol, urea
DT (Sanofi)	6/2018	aluminum phosphate, isotonic sodium chloride, formaldehyde
DTaP (Daptacel)	12/2018	aluminum phosphate, formaldehyde, glutaraldehyde, 2-phenoxyethanol
DTaP (Infanrix)	12/2018	formaldehyde, aluminum hydroxide, sodium chloride, polysorbate 80 (Tween 80)
DTaP-IPV (Kinrix)	12/2018	Formaldehyde, aluminum hydroxide, sodium chloride, polysorbate 80 (Tween 80), neomycin sulfate, polymyxin B
DTaP-IPV (Quadracel)	1/2019	formaldehyde, aluminum phosphate, 2-phenoxyethanol, polysorbate 80, glutaraldehyde, neomycin, polymyxin B sulfate, bovine serum albumin
DTaP-HepB-IPV (Pediarix)	2/2020*	formaldehyde, aluminum hydroxide, aluminum phosphate, sodium chloride, polysorbate 80 (Tween 80), neomycin sulfate, polymyxin B, yeast protein
DTaP-IPV/Hib (Pentacel)	1/2019	aluminum phosphate, polysorbate 80, sucrose, formaldehyde, glutaraldehyde, bovine serum albumin, 2-phenoxyethanol, neomycin, polymyxin B sulfate
DTaP-IPV-Hib-HepB (Vaxelis)	12/2018	polysorbate 80, formaldehyde, glutaraldehyde, bovine serum albumin, neomycin, streptomycin sulfate, polymyxin B sulfate, ammonium thiocyanate, yeast protein, aluminum
Ebola Zaire (ERVEBO)	2/2020*	Tromethamine rice-derived recombinant human serum albumin, host cell DNA benzonase, rice protein
Hib (ActHIB)	5/2019	sodium chloride, formaldehyde, sucrose
Hib (Hiberix)	4/2018	formaldehyde, sodium chloride, lactose

Vaccine	Date	Contains
Hib (PedvaxHIB)	10/2018	amorphous aluminum hydroxyphosphate sulfate, sodium chloride
Hep A (Havrix)	2/2020*	MRC-5 cellular proteins, formalin, aluminum hydroxide, amino acid supplement, phosphate-buffered saline solution, polysorbate 20, neomycin sulfate, aminoglycoside antibiotic
Hep A (Vaqta)	12/2018	amorphous aluminum hydroxyphosphate sulfate, non-viral protein, DNA, bovine albumin, formaldehyde, neomycin, sodium borate, sodium chloride, other process chemical residuals
Hep B (Engerix-B)	2/2020*	aluminum hydroxide, yeast protein, sodium chloride, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate
Hep B (Recombivax)	12/2018	formaldehyde, potassium aluminum sulfate, amorphous aluminum hydroxyphosphate sulfate, yeast protein
Hep B (Heplisav-B)	2017	yeast protein, yeast DNA, deoxycholate, phosphorothioate linked oligodeoxynucleotide, sodium phosphate, dibasic dodecahydrate, sodium chloride, monobasic dehydrate, polysorbate 80
Hep A/Hep B (Twinrix)	2/2020*	MRC-5 cellular proteins, formalin, aluminum phosphate, aluminum hydroxide, amino acids, sodium chloride, phosphate buffer, polysorbate 20, neomycin sulfate, yeast protein, water
Human Papillomavirus (HPV) (Gardasil 9)	10/2018	amorphous aluminum hydroxyphosphate sulfate, sodium chloride, L-histidine, polysorbate 80, sodium borate, yeast protein
Influenza (Afluria) Quadrivalent	12/2019	sodium chloride, monobasic sodium phosphate, dibasic sodium phosphate, monobasic potassium phosphate, potassium chloride, calcium chloride, sodium taurodeoxycholate, ovalbumin, sucrose, neomycin sulfate, polymyxin B, beta-propiolactone, hydrocortisone thimerosal (multi-dose vials)
Influenza (Fluad)	4/2019	squalene, polysorbate 80, sorbitan trioleate, sodium citrate dehydrate, citric acid monohydrate, neomycin, kanamycin, barium, hydrocortisone, egg proteins, cetyltrimethylammonium bromide (CTAB), formaldehyde
Influenza (Fluarix) Quadrivalent	©2019	octoxynol-10 (TRITON X-100), α-tocopheryl hydrogen succinate, polysorbate 80 (Tween 80), hydrocortisone, gentamicin sulfate, ovalbumin, formaldehyde, sodium deoxycholate, sodium phosphate-buffered isotonic sodium chloride
Influenza (Flublok) Quadrivalent	4/2019	sodium chloride, monobasic sodium phosphate, dibasic sodium phosphate, polysorbate 20 (Tween 20), baculovirus and *Spodoptera frugiperda* cell proteins, baculovirus and cellular DNA, Triton X-100
Influenza (Flucelvax) Quadrivalent	8/2019	Madin Darby Canine Kidney (MDCK) cell protein, phosphate buffered saline, protein other than HA, MDCK cell DNA, polysorbate 80, cetyltrimethlyammonium bromide, and β-propiolactone, Thimerosal (multi-dose vials)
Influenza (Flulaval) Quadrivalent	2/2020*	ovalbumin, formaldehyde, sodium deoxycholate, α-tocopheryl hydrogen succinate, polysorbate 80, thimerosal (multi-dose vials), phosphate-buffered saline solution
Influenza (Fluzone) Quadrivalent	2019	formaldehyde, egg protein, octylphenol ethoxylate (Triton X-100), sodium phosphate-buffered isotonic sodium chloride solution, thimerosal (multi-dose vials)
Influenza (Fluzone) High Dose	1/2019	egg protein, octylphenol ethoxylate (Triton X-100), sodium phosphate-buffered isotonic sodium chloride solution, formaldehyde
Influenza (FluMist) Quadrivalent	8/2019	monosodium glutamate, hydrolyzed porcine gelatin, arginine, sucrose, dibasic potassium phosphate, monobasic potassium phosphate, ovalbumin, gentamicin sulfate, ethylenediaminetetraacetic acid (EDTA)
Japanese Encephalitis (Ixiaro)	9/2018	aluminum hydroxide, protamine sulfate, formaldehyde, bovine serum albumin, Vero cell DNA, sodium metabisulphite, Vero cell protein
Meningococcal (MenACWY-Menactra)	4/26/18	sodium phosphate-buffered isotonic sodium chloride solution, formaldehyde, diphtheria toxoid
Meningococcal (MenACWY-Menveo)	2/2020*	formaldehyde, CRM_{197} protein
Meningococcal (MenB – Bexsero)	2/2020*	aluminum hydroxide, sodium chloride, histidine, sucrose, kanamycin
Meningococcal (MenB – Trumenba)	2018	polysorbate 80, aluminum phosphate, histidine buffered saline

Vaccine	Date	Contains
MMR (MMR-II)	2/2020*	vitamins, amino acids, fetal bovine serum, sucrose, glutamate, recombinant human albumin, neomycin, sorbitol, hydrolyzed gelatin, sodium phosphate, sodium chloride, WI-38 human diploid lung fibroblasts
MMRV (ProQuad) (Frozen: Recombinant Albumin)	2/2020*	MRC-5 cells including DNA and protein, sucrose, hydrolyzed gelatin, sodium chloride, sorbitol, monosodium L-glutamate, sodium phosphate dibasic, recombinant human albumin, sodium bicarbonate, potassium phosphate monobasic, potassium chloride; potassium phosphate dibasic, neomycin, bovine calf serum
MMRV (ProQuad) (Frozen: Human Serum Albumin)	2/2020*	MRC-5 cells including DNA and protein, sucrose, hydrolyzed gelatin, sodium chloride, sorbitol, monosodium L-glutamate, sodium phosphate dibasic, human albumin, sodium bicarbonate, potassium phosphate monobasic, potassium chloride; potassium phosphate dibasic, neomycin, bovine calf serum
MMRV (ProQuad) (Refrigerator Stable)	10/2018	MRC-5 cells including DNA and protein, sucrose, hydrolyzed gelatin, urea, sodium chloride, sorbitol, monosodium L-glutamate, sodium phosphate, recombinant human albumin, sodium bicarbonate, potassium phosphate, potassium chloride, neomycin, bovine serum albumin
Pneumococcal (PCV13 – Prevnar 13)	8/2017	CRM_{197} carrier protein, polysorbate 80, succinate buffer, aluminum phosphate
Pneumococcal (PPSV-23 – Pneumovax)	2/2020*	isotonic saline solution, phenol
Polio (IPV – Ipol)	2/2020*	calf bovine serum albumin, 2-phenoxyethanol, formaldehyde, neomycin, streptomycin, polymyxin B, M-199 medium
Rabies (Imovax)	10/2019	human albumin, neomycin sulfate, phenol red, beta-propiolactone
Rabies (RabAvert)	©2018	chicken protein, polygeline (processed bovine gelatin), human serum albumin, potassium glutamate, sodium EDTA, ovalbumin, neomycin, chlortetracycline, amphotericin B
Rotavirus (RotaTeq)	2/2017	sucrose, sodium citrate, sodium phosphate monobasic monohydrate, sodium hydroxide, polysorbate 80, cell culture media, fetal bovine serum *[DNA from porcine circoviruses (PCV) 1 and 2 has been detected in RotaTeq. PCV-1 and PCV-2 are not known to cause disease in humans.]*
Rotavirus (Rotarix)	2/2020*	dextran, Dulbecco's Modified Eagle Medium (sodium chloride, potassium chloride, magnesium sulfate, ferric (III) nitrate, sodium phosphate, sodium pyruvate, D-glucose, concentrated vitamin solution, L-cystine, L-tyrosine, amino acids, L-glutamine, calcium chloride, sodium hydrogenocarbonate, and phenol red), sorbitol, sucrose, calcium carbonate, sterile water, xanthan *[Porcine circovirus type 1 (PCV-1) is present in Rotarix. PCV-1 is not known to cause disease in humans.]*
Smallpox (Vaccinia) (ACAM2000)	3/2018	HEPES, 2% human serum albumin, 0.5 - 0.7% sodium chloride USP, 5% Mannitol USP, neomycin, polymyxin B, 50% Glycerin USP, 0.25% phenol USP
Td (Tenivac)	11/2019	aluminum phosphate, formaldehyde, sodium chloride, water
Td (TDVAX)	9/2018	aluminum phosphate, formaldehyde, thimerosal
Tdap (Adacel)	1/2019	aluminum phosphate, formaldehyde, 2-phenoxyethanol, glutaraldehyde, water
Tdap (Boostrix)	2/2020*	formaldehyde, aluminum hydroxide, sodium chloride, polysorbate 80
Typhoid (Typhim Vi)	3/2014	formaldehyde, phenol, polydimethylsiloxane, disodium phosphate, monosodium phosphate, sodium chloride, sterile water
Typhoid (Vivotif Ty21a)	9/2013	sucrose, ascorbic acid, amino acids, lactose, magnesium stearate. gelatin
Varicella (Varivax) *Frozen*	2/2020*	MRC-5 human diploid cells, including DNA & protein, sucrose, hydrolyzed gelatin, sodium chloride, monosodium L-glutamate, sodium phosphate dibasic, sodium phosphate monobasic, potassium phosphate monobasic, potassium chloride, EDTA, neomycin, fetal bovine serum
Varicella (Varivax) *Refrigerator Stable*	10/2018	MRC-5 human diploid cells, including DNA & protein, sucrose, hydrolyzed gelatin, sodium chloride, monosodium L-glutamate, urea, sodium phosphate dibasic, potassium phosphate monobasic, potassium chloride, neomycin, bovine calf serum
Yellow Fever (YF-Vax)	2/2019	sorbitol, gelatin, sodium chloride
Zoster (Shingles) (Zostavax) *Frozen*	1/2019	MRC-5 human diploid cells, including DNA & protein, sucrose, hydrolyzed porcine gelatin, sodium chloride, monosodium L-glutamate, sodium phosphate dibasic, potassium phosphate monobasic, potassium chloride; neomycin, bovine calf serum

Vaccine	Date	Contains
Zoster (Shingles) (Zostavax) *Refrigerator Stable*	8/2018	MRC-5 human diploid cells, including DNA & protein, sucrose, hydrolyzed porcine gelatin, urea, sodium chloride, monosodium L-glutamate, sodium phosphate dibasic, potassium phosphate monobasic, potassium chloride, neomycin, bovine calf serum
Zoster (Shingles) (Shingrix)	2/2020*	sucrose, sodium chloride, dioleoyl phosphatidylcholine (DOPC), 3-*O*-desacl-4'monophosphoryl lipid A (MPL), QS-21 (a saponin purified from plant extract *Quillaja saponaria* Molina), potassium dihydrogen phosphate, cholesterol, sodium dihydrogen phosphate dihydrate, disodium phosphate anhydrous, dipotassium phosphate, polysorbate 80, host cell protein and DNA

A table listing vaccine excipients and media *by excipient* is published by the Institute for Vaccine Safety at Johns Hopkins University, and can be found at http://www.vaccinesafety.edu/components-Excipients.htm.

February 2020

EXHIBIT 353

Common Ingredients in U.S. Licensed Vaccines

The vast majority of the over one billion doses of vaccines manufactured worldwide each year are given to healthy babies, children and adults. Thus, it is critical that vaccines be demonstrated to be safe and effective. FDA requires that vaccines undergo a rigorous and extensive development program in the laboratory, as well as in animal studies and human clinical trials, to determine their safety and effectiveness. Highly trained FDA scientists and clinicians carefully evaluate all of the information in a marketing application and make a determination whether to license (approve) a vaccine before it can be used in the United States. Prior to licensure, as part of FDA's evaluation, FDA takes all of the ingredients of a vaccine into account, including the active ingredients as well as other substances. After FDA approves a vaccine, FDA continuously monitors its safety.

Why is aluminum in some vaccines?

Aluminum salts are incorporated into some vaccine formulations as an adjuvant. An adjuvant is a substance added to some vaccines to enhance the immune response of vaccinated individuals. The aluminum salts in some U.S. licensed vaccines are aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), or mixed aluminum salts. For example: aluminum salts are used in DTaP vaccines, the pneumococcal conjugate vaccine, and hepatitis B vaccines.

Aluminum adjuvant containing vaccines have a demonstrated safety profile of over six decades of use and have only uncommonly been associated with severe local reactions. A study conducted by FDA (https://wayback.archive-it.org/7993/20170405003134/https:/www.fda.gov/BiologicsBloodVaccines/ScienceResearch/ucm284520. (http://www.fda.gov/about-fda/website-policies/website-disclaimer) determined that the risk to infants posed by the total aluminum exposure received from the entire recommended series of childhood vaccines over the first year of life is extremely low. This study provided additional scientific information confirming that the benefits of aluminum-containing vaccines administered during the first year of life outweigh any theoretical concerns about the potential effect of aluminum on infants. Of note, the most common source of exposure to aluminum is from eating food or drinking water.

Are other adjuvants used in FDA-approved vaccines?

Yes. Cervarix, a vaccine to prevent cervical cancer caused by human papillomavirus types 16 and 18, includes AS04 in its formulation. AS04 is a combination of aluminum hydroxide and monophosphoryl lipid A (MPL). MPL is a purified fat-like substance. The manufacturer no longer markets Cervarix in the United States.

One vaccine for the prevention of H5N1 influenza, commonly referred to as avian influenza or "bird flu," contains the adjuvant AS03, an oil-in-water emulsion. The AS03 adjuvant is made up of the oily compounds, D,L-alpha-tocopherol (vitamin E) and squalene (/vaccines-blood-biologics/vaccine-safety-availability/influenza-h5n1-virus-monovalent-vaccine-adjuvanted-manufactured-id-biomedical-corporation-questions#squalene), and an emulsifier, polysorbate 80, which helps ingredients mix together and keep them from separating, and water containing small amounts of salts. The vaccine is not commercially available, but included within the U.S. government's National Stockpile if public health officials determine it is needed.

Fluad, a vaccine for the prevention of seasonal influenza in adults 65 years of age and older, includes MF59, also an oil-in-water emulsion of squalene (/vaccines-blood-biologics/vaccine-safety-availability/influenza-h5n1-virus-monovalent-vaccine-adjuvanted-manufactured-id-biomedical-corporation-questions#squalene) oil.

Heplisav-B, a vaccine for the prevention of infection caused hepatitis B virus in adults 18 years of age and older, includes CpG 1018, an adjuvant based on synthetic DNA sequences.

Shingrix, a vaccine for the prevention of shingles in adults 50 years of age and older, includes AS01B. AS01B is made of up MPL, a purified fat-like substance, and QS-21 which is purified from the bark of the Quillaja saponaria (soap bark) evergreen tree native to central Chile.

How does FDA evaluate adjuvants for safety and efficacy?

When evaluating a vaccine for safety and efficacy, FDA considers adjuvants as a component of the vaccine; they are not licensed separately.

Why are antibiotics in some vaccines?

Certain antibiotics may be used in some vaccine production to help prevent bacterial contamination during manufacturing. As a result, small amounts of antibiotics may be present in some vaccines. Because some antibiotics can cause severe allergic reactions in those children allergic to them (such as hives, swelling at the back of the throat, and low blood pressure), some parents are concerned that antibiotics contained in vaccines might be harmful. However, antibiotics most likely to cause severe allergic reactions (e.g., penicillins, cephalosporins and sulfa drugs) are not used in vaccine production, and therefore are not contained in vaccines.

Examples of antibiotics used during vaccine manufacture include neomycin, polymyxin B, streptomycin and gentamicin. Some antibiotics used in vaccine production are present in the vaccine, either in very small amounts or they are undetectable. For example, antibiotics are used in some production methods for

making inactivated influenza virus vaccines. They are used to reduce bacterial growth in eggs during processing steps, because eggs are not sterile products. The antibiotics that are used are reduced to very small or undetectable amounts during subsequent purification steps. The very small amounts of antibiotics contained in vaccines have not been clearly associated with severe allergic reactions.

Why is formaldehyde in some vaccines?

Formaldehyde has a long history of safe use in the manufacture of certain viral and bacterial vaccines. It is used to inactivate viruses so that they don't cause disease (e.g., polio virus used to make polio vaccine) and to detoxify bacterial toxins, such as the toxin used to make diphtheria vaccine. Formaldehyde is diluted during the vaccine manufacturing process, but residual quantities of formaldehyde may be found in some current vaccines. The amount of formaldehyde present in some vaccines is so small compared to the concentration that occurs naturally in the body that it does not pose a safety concern.

Formaldehyde is also produced naturally in the human body as a part of normal functions of the body to produce energy and build the basic materials needed for important life processes. This includes making amino acids, which are the building blocks of proteins that the body needs.

Formaldehyde is also found in the environment and is present in different ways. It is used in building materials, as a preservative in labs and to produce many household products.

The body continuously processes formaldehyde, both from what it makes on its own and from what it has been exposed to in the environment. When the body breaks down formaldehyde, it does not distinguish between formaldehyde from vaccines and that which is naturally produced or environmental. The amount of formaldehyde in a person's body depends on their weight; babies have lower amounts than adults. Studies have shown that for a newborn of average weight of 6 - 8 pounds, the amount of formaldehyde in their body is 50-70 times higher than the upper amount that they could receive from a single dose of a vaccine or from vaccines administered over time.

Excessive exposure to formaldehyde may cause cancer, but the latest research has shown that the highest risk is from the air when formaldehyde is inhaled from breathing, and occurs more frequently in people who routinely use formaldehyde in their jobs. There is no evidence linking cancer to infrequent exposure to tiny amounts of formaldehyde via injection as occurs with vaccines.

Why are sugars, amino acids, and proteins added to some vaccines?

These substances may be added as stabilizers. They help protect the vaccine from adverse conditions such as the freeze-drying process, for those vaccines that are freeze dried. Stabilizers added to vaccines include: sugars such as sucrose and lactose, amino acids such as glycine or the monosodium salt of glutamic acid and proteins such as human serum albumin or gelatin. Sugars, amino acids and proteins are not unique to vaccines and are encountered in everyday life in the diet and are components that are in the body naturally.

Why are there preservatives in some vaccines?

Preservatives are added to some vaccine formulations to prevent the growth of bacteria or fungi that may be introduced into the vaccine during its use, e.g., repeated puncture of a multi-dose vaccine vial with a needle.

Why is fetal calf/bovine serum in some vaccines?

In the manufacture of viral vaccines, the virus may be grown in cells. These cells need a source of nutrition, which in some instances may be provided by fetal bovine serum.

EXHIBIT 354

ATSDR Agency for Toxic Substances & Disease Registry

Substances A-Z

Search Substances by Chemical Abstracts Service Number (CAS#), Substance Name, Synonym, or Tradename.

Enter Search Criteria*

Search

**Full or Partial Spelling*

With one click, access the best science, the latest research, and the most important information about toxic substances and how they affect our health including:

- Characteristics
- Exposure risks
- Associated health effects
- Related CDC and ATSDR health studies and assessments

Substances A-Z

A B C D E F G H I J L M N O P R S T U V W X Z

A

- Acetone (toxsubstance.asp?toxid=1)
- Acrolein (toxsubstance.asp?toxid=102)
- Acrylamide (toxsubstance.asp?toxid=236)
- Acrylonitrile (toxsubstance.asp?toxid=78)
- Aldrin/Dieldrin (toxsubstance.asp?toxid=56)
- Aluminum (toxsubstance.asp?toxid=34)
- Americium (toxsubstance.asp?toxid=158)
- Ammonia (toxsubstance.asp?toxid=2)
- Aniline (toxsubstance.asp?toxid=79)
- Antimony (toxsubstance.asp?toxid=58)
- Arsenic (toxsubstance.asp?toxid=3)
- Arsine (toxsubstance.asp?toxid=278)
- Asbestos (toxsubstance.asp?toxid=4)
- Atrazine (toxsubstance.asp?toxid=59)

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B

- 1,3-Butadiene (toxsubstance.asp?toxid=81)
- 1-Bromopropane (toxsubstance.asp?toxid=285)
- 2,3-Benzofuran (toxsubstance.asp?toxid=187)
- 2-Butanone (toxsubstance.asp?toxid=60)
- 2-Butoxyethanol and 2-Butoxyethanol Acetate (toxsubstance.asp?toxid=61)
- Barium (toxsubstance.asp?toxid=57)
- Benzene (toxsubstance.asp?toxid=14)
- Benzidine (toxsubstance.asp?toxid=105)
- Beryllium (toxsubstance.asp?toxid=33)
- Bis(2-chloroethyl) Ether (toxsubstance.asp?toxid=159)
- Bis(chloromethyl) Ether (toxsubstance.asp?toxid=188)
- Blister Agents HN-1HN-2HN-3 Nitrogen Mustards (toxsubstance.asp?toxid=189)
- Blister Agents: Lewisite (L), Mustard-Lewisite Mixture (HL) (toxsubstance.asp?toxid=190)
- Blister Agents: Sulfur Mustard Agent H/HD, Sulfur Mustard Agent HT (toxsubstance.asp?toxid=191)
- Boron (toxsubstance.asp?toxid=80)
- Bromodichloromethane (toxsubstance.asp?toxid=127)
- Bromoform & Dibromochloromethane (toxsubstance.asp?toxid=128)
- Bromomethane (toxsubstance.asp?toxid=160)

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C

- Cadmium (toxsubstance.asp?toxid=15)
- Calcium Hypochlorite/Sodium Hypochlorite (toxsubstance.asp?toxid=192)
- Carbon Disulfide (toxsubstance.asp?toxid=84)
- Carbon Monoxide (toxsubstance.asp?toxid=253)

- Carbon Tetrachloride (toxsubstance.asp?toxid=35)
- Cesium (toxsubstance.asp?toxid=107)
- Chlordane (toxsubstance.asp?toxid=62)
- Chlordecone (toxsubstance.asp?toxid=118)
- Chlorfenvinphos (toxsubstance.asp?toxid=193)
- Chlorinated Dibenzo-p-dioxins (CDDs) (toxsubstance.asp?toxid=63)
- Chlorine (toxsubstance.asp?toxid=36)
- Chlorine Dioxide & Chlorite (toxsubstance.asp?toxid=108)
- Chlorobenzene (toxsubstance.asp?toxid=87)
- Chlorodibenzofurans (CDFs) (toxsubstance.asp?toxid=194)
- Chloroethane (toxsubstance.asp?toxid=161)
- Chloroform (toxsubstance.asp?toxid=16)
- Chloromethane (toxsubstance.asp?toxid=109)
- Chlorophenols (toxsubstance.asp?toxid=195)
- Chlorpyrifos (toxsubstance.asp?toxid=88)
- Chromium (toxsubstance.asp?toxid=17)
- Cobalt (toxsubstance.asp?toxid=64)
- Copper (toxsubstance.asp?toxid=37)
- Creosote (toxsubstance.asp?toxid=18)
- Cresols (toxsubstance.asp?toxid=196)
- Crotonaldehyde (toxsubstance.asp?toxid=197)
- Cyanide (toxsubstance.asp?toxid=19)

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D

- 1,1-Dichloroethane (toxsubstance.asp?toxid=129)
- 1,1-Dichloroethene (toxsubstance.asp?toxid=130)
- 1,2-Dibromo-3-Chloropropane (toxsubstance.asp?toxid=166)
- 1,2-Dibromoethane (toxsubstance.asp?toxid=131)
- 1,2-Dichloroethane (toxsubstance.asp?toxid=110)
- 1,2-Dichloroethene (toxsubstance.asp?toxid=82)
- 1,2-Dichloropropane (toxsubstance.asp?toxid=162)
- 1,2-Diphenylhydrazine (toxsubstance.asp?toxid=198)
- 1,3 Dinitrobenzene & 1,3,5 Trinitrobenzene (toxsubstance.asp?toxid=164)
- 1,4-Dioxane (toxsubstance.asp?toxid=199)
- 2,4-Dichlorophenoxyacetic Acid (2,4-D) (toxsubstance.asp?toxid=288)
- 3,3'-Dichlorobenzidine (toxsubstance.asp?toxid=200)
- DDT, DDE, DDD (toxsubstance.asp?toxid=20)
- DEET (N,N-diethyl-meta-toluamide) (toxsubstance.asp?toxid=201)
- Di(2-Ethylhexyl)Phthalate (DEHP) (toxsubstance.asp?toxid=65)
- Di-n-butyl Phthalate (toxsubstance.asp?toxid=167)
- Di-n-octylphthalate (DNOP) (toxsubstance.asp?toxid=204)
- Diazinon (toxsubstance.asp?toxid=90)
- Diborane (toxsubstance.asp?toxid=202)
- Dichlorobenzenes (toxsubstance.asp?toxid=126)
- Dichloropropenes (toxsubstance.asp?toxid=163)

- Dichlorvos (toxsubstance.asp?toxid=111)
- Diethyl phthalate (toxsubstance.asp?toxid=112)
- Diisopropyl Methylphosphonate (DIMP) (toxsubstance.asp?toxid=203)
- Dinitrocresols (toxsubstance.asp?toxid=218)
- Dinitrophenols (toxsubstance.asp?toxid=132)
- Dinitrotoluenes (toxsubstance.asp?toxid=165)
- Disulfoton (toxsubstance.asp?toxid=205)

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E

- Endosulfan (toxsubstance.asp?toxid=113)
- Endrin (Endrin aldehyde) (toxsubstance.asp?toxid=114)
- Ethion (toxsubstance.asp?toxid=206)
- Ethylbenzene (toxsubstance.asp?toxid=66)
- Ethylene Dibromide (toxsubstance.asp?toxid=251)
- Ethylene Glycol (toxsubstance.asp?toxid=21)
- Ethylene Oxide (toxsubstance.asp?toxid=133)

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F

- Fluorides, Hydrogen Fluoride, and Fluorine (toxsubstance.asp?toxid=38)
- Formaldehyde (toxsubstance.asp?toxid=39)
- Fuel Oils / Kerosene (toxsubstance.asp?toxid=91)

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G

- Gasoline, Automotive (toxsubstance.asp?toxid=83)
- Glutaraldehyde (toxsubstance.asp?toxid=284)
- Glyphosate (toxsubstance.asp?toxid=293)
- Guthion (toxsubstance.asp?toxid=207)

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H

- 2-Hexanone (toxsubstance.asp?toxid=134)
- Heptachlor/Heptachlor Epoxide (toxsubstance.asp?toxid=135)
- Hexachlorobenzene (toxsubstance.asp?toxid=115)
- Hexachlorobutadiene (toxsubstance.asp?toxid=168)
- Hexachlorocyclohexane (HCH) (toxsubstance.asp?toxid=138)
- Hexachlorocyclopentadiene (HCCPD) (toxsubstance.asp?toxid=208)
- Hexachloroethane (toxsubstance.asp?toxid=169)
- Hexamethylene Diisocyanate (HDI) (toxsubstance.asp?toxid=170)
- HMX (Octogen) (toxsubstance.asp?toxid=171)

- Hydraulic Fluids (toxsubstance.asp?toxid=141)
- Hydrazines (toxsubstance.asp?toxid=89)
- Hydrogen Chloride (toxsubstance.asp?toxid=147)
- Hydrogen Cyanide (HCN) (toxsubstance.asp?toxid=249)
- Hydrogen Fluoride (HF) (toxsubstance.asp?toxid=250)
- Hydrogen Peroxide (toxsubstance.asp?toxid=55)
- Hydrogen Sulfide Carbonyl Sulfide (toxsubstance.asp?toxid=67)
- n-Hexane (toxsubstance.asp?toxid=68)

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I

- Iodine (toxsubstance.asp?toxid=85)
- Ionizing Radiation (toxsubstance.asp?toxid=86)
- Isophorone (toxsubstance.asp?toxid=148)

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J

- Jet Fuels JP-4 and JP-7 (toxsubstance.asp?toxid=149)
- JP-5, JP-8, and Jet A (toxsubstance.asp?toxid=150)

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L

- Lead (toxsubstance.asp?toxid=22)

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M

- 4,4'-Methylenebis(2-Chloroaniline) (MBOCA) (toxsubstance.asp?toxid=209)
- 4,4'-Methylenedianiline (toxsubstance.asp?toxid=210)
- Malathion (toxsubstance.asp?toxid=92)
- Manganese (toxsubstance.asp?toxid=23)
- Mercury (toxsubstance.asp?toxid=24)
- Methoxychlor (toxsubstance.asp?toxid=151)
- Methyl Isocyanate (toxsubstance.asp?toxid=116)
- Methyl Mercaptan (toxsubstance.asp?toxid=40)
- Methyl Parathion (toxsubstance.asp?toxid=117)
- Methyl tert-Butyl Ether (MTBE) (toxsubstance.asp?toxid=41)
- Methylene Chloride (toxsubstance.asp?toxid=42)
- Mirex (toxsubstance.asp?toxid=276)
- Molybdenum (toxsubstance.asp?toxid=289)

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N

- n-Nitrosodi-n-propylamine (toxsubstance.asp?toxid=211)
- n-Nitrosodimethylamine (toxsubstance.asp?toxid=173)
- n-Nitrosodiphenylamine (toxsubstance.asp?toxid=212)
- Naphthalene, 1-Methylnapthalene, 2-Methylnapthalene (toxsubstance.asp?toxid=43)
- Nerve Agents (GA, GB, GD, VX) (toxsubstance.asp?toxid=93)
- Nickel (toxsubstance.asp?toxid=44)
- Nitrate and Nitrite (toxsubstance.asp?toxid=258)
- Nitrobenzene (toxsubstance.asp?toxid=95)
- Nitrogen Oxides (toxsubstance.asp?toxid=69)
- Nitrophenols (toxsubstance.asp?toxid=172)

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O

- Otto Fuel II and its Components (toxsubstance.asp?toxid=152)

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P

- Parathion (toxsubstance.asp?toxid=246)
- Pentachlorophenol (toxsubstance.asp?toxid=70)
- Perchlorates (toxsubstance.asp?toxid=181)
- Perfluoroalkyls (toxsubstance.asp?toxid=237)
- Phenol (toxsubstance.asp?toxid=27)
- Phosgene (toxsubstance.asp?toxid=182)
- Phosgene Oxime (toxsubstance.asp?toxid=213)
- Phosphate Ester Flame Retardants (toxsubstance.asp?toxid=239)
- Phosphine (toxsubstance.asp?toxid=214)
- Plutonium (toxsubstance.asp?toxid=119)
- Polybrominated Biphenyls (PBBs) (toxsubstance.asp?toxid=94)
- Polybrominated Diphenyl Ethers (PBDEs) (toxsubstance.asp?toxid=183)
- Polychlorinated Biphenyls (PCBs) (toxsubstance.asp?toxid=26)
- Polycyclic Aromatic Hydrocarbons (PAHs) (toxsubstance.asp?toxid=25)
- Propylene Glycol (toxsubstance.asp?toxid=240)
- Pyrethrins and Pyrethroids (toxsubstance.asp?toxid=153)
- Pyridine (toxsubstance.asp?toxid=96)

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R

- Radium (toxsubstance.asp?toxid=154)
- Radon (toxsubstance.asp?toxid=71)
- RDX (Cyclonite) (toxsubstance.asp?toxid=72)

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S

- S,S,S-Tributyl Phosphorotrithioate (toxsubstance.asp?toxid=292)
- Selenium (toxsubstance.asp?toxid=28)
- Selenium Hexafluoride (toxsubstance.asp?toxid=215)
- Silica (toxsubstance.asp?toxid=290)
- Silver (toxsubstance.asp?toxid=97)
- Sodium Hydroxide (toxsubstance.asp?toxid=45)
- Stoddard Solvent (toxsubstance.asp?toxid=73)
- Strontium (toxsubstance.asp?toxid=120)
- Styrene (toxsubstance.asp?toxid=74)
- Sulfur Dioxide (toxsubstance.asp?toxid=46)
- Sulfur Mustard (toxsubstance.asp?toxid=184)
- Sulfur Trioxide & Sulfuric Acid (toxsubstance.asp?toxid=47)
- Synthetic Vitreous Fibers (toxsubstance.asp?toxid=185)

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T

- 1,1,1-Trichloroethane (toxsubstance.asp?toxid=76)
- 1,1,2,2-Tetrachloroethane (toxsubstance.asp?toxid=156)
- 1,1,2-Trichloroethane (toxsubstance.asp?toxid=155)
- 1,2,3 Trichloropropane (toxsubstance.asp?toxid=186)
- 1,3,5 Trinitrobenzene & 1,3 Dinitrobenzene (toxsubstance.asp?toxid=241)
- 2,4,6-Trinitrotoluene (TNT) (toxsubstance.asp?toxid=125)
- Tetrachloroethylene (PERC) (toxsubstance.asp?toxid=48)
- Tetryl (toxsubstance.asp?toxid=216)
- Thallium (toxsubstance.asp?toxid=49)
- Thorium (toxsubstance.asp?toxid=121)
- Tin and Compounds (toxsubstance.asp?toxid=98)
- Titanium Tetrachloride (toxsubstance.asp?toxid=122)
- Toluene (toxsubstance.asp?toxid=29)
- Toluene Diisocyanate Methylenediphenyl Diisocyanate (toxsubstance.asp?toxid=245)
- Total Petroleum Hydrocarbons (TPH) (toxsubstance.asp?toxid=75)
- Toxaphene (toxsubstance.asp?toxid=99)
- Trichlorobenzenes (toxsubstance.asp?toxid=255)
- Trichloroethylene (TCE) (toxsubstance.asp?toxid=30)
- Tungsten (toxsubstance.asp?toxid=157)

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U

- Unidentified Chemical (toxsubstance.asp?toxid=243)
- Uranium (toxsubstance.asp?toxid=77)
- Used Mineral-based Crankcase Oil (toxsubstance.asp?toxid=123)

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V

- Vanadium (toxsubstance.asp?toxid=50)
- Vinyl Acetate (toxsubstance.asp?toxid=124)
- Vinyl Chloride (toxsubstance.asp?toxid=51)

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W

- White Phosphorus (toxsubstance.asp?toxid=52)

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X

- Xylenes (toxsubstance.asp?toxid=53)

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Z

- Zinc (toxsubstance.asp?toxid=54)

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- Page last reviewed: March 3, 2011
- Page last updated: March 3, 2011
- Content source: Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov)

Agency for Toxic Substances and Disease Registry, 4770 Buford Hwy NE, Atlanta, GA 30341
Contact CDC: 800-232-4636 / TTY: 888-232-6348

EXHIBIT 355

Vaccines Licensed for Use in the United States

Product Name	Trade Name
Adenovirus Type 4 and Type 7 Vaccine, Live, Oral (/vaccines-blood-biologics/approved-products/adenovirus-type-4-and-type-7-vaccine-live-oral)	No Trade Name
Anthrax Vaccine Adsorbed (/vaccines-blood-biologics/approved-products/anthrax-vaccine-adsorbed)	Biothrax
BCG Live (/vaccines-blood-biologics/approved-products/bcg-live)	BCG Vaccine
BCG Live (/vaccines-blood-biologics/approved-products/bcg-live)	TICE BCG
Cholera Vaccine Live Oral (/vaccines-blood-biologics/approved-products/vaxchora)	Vaxchora
Dengue Tetravalent Vaccine, Live (/vaccines-blood-biologics/dengvaxia)	DENGVAXIA
Diphtheria & Tetanus Toxoids Adsorbed (/vaccines-blood-biologics/approved-products/diphtheria-and-tetanus-toxoids-adsorbed)	No Trade Name
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed (/vaccines-blood-biologics/approved-products/infanrix)	Infanrix
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed (/vaccines-blood-biologics/approved-products/daptacel)	DAPTACEL
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed, Hepatitis B (recombinant) and Inactivated Poliovirus Vaccine Combined (/vaccines-blood-biologics/approved-products/diphtheria-and-	Pediarix

tetanus-toxoids-and-acellular-pertussis-adsorbed-hepatitis-b-recombinant-and)	
Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine (/vaccines-blood-biologics/approved-products/diphtheria-and-tetanus-toxoids-and-acellular-pertussis-adsorbed-and-inactivated-poliovirus-vaccine)	KINRIX
Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine (/vaccines-blood-biologics/approved-products/diphtheria-and-tetanus-toxoids-and-acellular-pertussis-adsorbed-and-inactivated-poliovirus-vaccine)	Quadracel
Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus, Haemophilus b Conjugate [Meningococcal Protein Conjugate] and Hepatitis B [Recombinant] Vaccine (/vaccines-blood-biologics/vaxelis)	VAXELIS
Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine (/vaccines-blood-biologics/approved-products/diphtheria-and-tetanus-toxoids-and-acellular-pertussis-adsorbed-inactivated-poliovirus-and)	Pentacel
Ebola Zaire Vaccine, Live (/vaccines-blood-biologics/ervebo)	ERVEBO
Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate) (/vaccines-blood-biologics/approved-products/haemophilus-b-conjugate-vaccine-meningococcal-protein-conjugate)	PedvaxHIB
Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate) (/vaccines-blood-biologics/approved-products/haemophilus-b-conjugate-vaccine-tetanus-toxoid-conjugate)	ActHIB
Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate) (/vaccines-blood-biologics/approved-products/hiberix)	Hiberix
Hepatitis A Vaccine, Inactivated (/vaccines-blood-	Havrix

biologics/approved-products/havrix)	
Hepatitis A Vaccine, Inactivated (/vaccines-blood-biologics/approved-products/vaqta)	VAQTA
Hepatitis A Inactivated and Hepatitis B (Recombinant) Vaccine (/vaccines-blood-biologics/approved-products/twinrix)	Twinrix
Hepatitis B Vaccine (Recombinant) (/vaccines-blood-biologics/approved-products/recombivax-hb)	Recombivax HB
Hepatitis B Vaccine (Recombinant) (/vaccines-blood-biologics/approved-products/engerix-b)	Engerix-B
Hepatitis B Vaccine (Recombinant), Adjuvanted (/vaccines-blood-biologics/approved-products/heplisav-b)	HEPLISAV-B
Human Papillomavirus Quadrivalent (Types 6, 11, 16, 18) Vaccine, Recombinant (/vaccines-blood-biologics/approved-products/human-papillomavirus-vaccine)	Gardasil
Human Papillomavirus 9-valent Vaccine, Recombinant (/vaccines-blood-biologics/approved-products/gardasil-9)	Gardasil 9
Human Papillomavirus Bivalent (Types 16, 18) Vaccine, Recombinant (/vaccines-blood-biologics/approved-products/cervarix)	Cervarix
Influenza A (H1N1) 2009 Monovalent Vaccine (/vaccines-blood-biologics/approved-products/influenza-h1n1-2009-monovalent-vaccine-csl-limited)	No Trade Name
Influenza A (H1N1) 2009 Monovalent Vaccine (/vaccines-blood-biologics/approved-products/influenza-h1n1-2009-monovalent-vaccine-medimmune-llc)	No Trade Name
Influenza A (H1N1) 2009 Monovalent Vaccine (/vaccines-blood-biologics/approved-products/influenza-h1n1-2009-monovalent-vaccine-id-biomedical-corporation-quebec)	No Trade Name

Influenza A (H1N1) 2009 Monovalent Vaccine (/vaccines-blood-biologics/approved-products/influenza-h1n1-2009-monovalent-vaccine-novartis-vaccines-and-diagnostics-limited)	No Trade Name
Influenza A (H1N1) 2009 Monovalent Vaccine (/vaccines-blood-biologics/approved-products/influenza-h1n1-2009-monovalent-vaccine-sanofi-pasteur-inc)	No Trade Name
Influenza Virus Vaccine, H5N1 (/vaccines-blood-biologics/approved-products/influenza-virus-vaccine-h5n1-national-stockpile) (for National Stockpile)	No Trade Name
Influenza A (H5N1) Virus Monovalent Vaccine, Adjuvanted (/vaccines-blood-biologics/approved-products/influenza-h5n1-virus-monovalent-vaccine-adjuvanted)	No Trade Name
Influenza A (H5N1) Monovalent Vaccine, Adjuvanted (/vaccines-blood-biologics/audenz)	AUDENZ
Influenza Vaccine, Adjuvanted (/vaccines-blood-biologics/fluad-quadrivalent)	FLUAD QUADRIVALENT
Influenza Vaccine, Adjuvanted (/vaccines-blood-biologics/approved-products/fluad)	FLUAD
Influenza Vaccine (/vaccines-blood-biologics/approved-products/afluria-quadrivalent)	AFLURIA QUADRIVALENT
Influenza Vaccine (/vaccines-blood-biologics/approved-products/flucelvax-quadrivalent)	Flucelvax Quadrivalent
Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/afluria)	Afluria
Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/flulaval)	FluLaval
Influenza Vaccine, Live, Intranasal (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/flumist)	FluMist

Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/fluarix)	Fluarix
Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/fluvirin)	Fluvirin
Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/agriflu)	Agriflu
Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/fluzone-fluzone-high-dose-and-fluzone-intradermal)	Fluzone, Fluzone High-Dose and Fluzone Intradermal
Influenza Virus Vaccine (Trivalent, Types A and B) (/vaccines-blood-biologics/approved-products/flucelvax)	Flucelvax
Influenza Vaccine (Trivalent) (/vaccines-blood-biologics/approved-products/flublok)	Flublok
Influenza Vaccine (Quadrivalent) (/vaccines-blood-biologics/approved-products/flublok-quadrivalent)	Flublok Quadrivalent
Influenza Vaccine,Live, Intranasal (Quadrivalent, Types A and Types B) (/vaccines-blood-biologics/approved-products/flumist-quadrivalent)	FluMist Quadrivalent
Influenza Virus Vaccine (Quadrivalent, Types A and Types B) (/vaccines-blood-biologics/approved-products/fluarix-quadrivalent)	Fluarix Quadrivalent
Influenza Virus Vaccine (Quadrivalent, Types A and Types B) (/vaccines-blood-biologics/approved-products/fluzone-quadrivalent)	Fluzone Quadrivalent
Influenza Vaccine (/vaccines-blood-biologics/approved-products/flulaval-quadrivalent)	Flulaval Quadrivalent
Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed (/vaccines-blood-biologics/approved-products/japanese-encephalitis-vaccine-inactivated-	Ixiaro

adsorbed)	
Measles, Mumps, and Rubella Virus Vaccine, Live (/vaccines-blood-biologics/approved-products/measles-mumps-and-rubella-virus-vaccine-live)	M-M-R II
Measles, Mumps, Rubella and Varicella Virus Vaccine Live (/vaccines-blood-biologics/approved-products/measles-mumps-rubella-and-varicella-virus-vaccine-live)	ProQuad
Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM197 Conjugate Vaccine (/vaccines-blood-biologics/approved-products/menveo)	Menveo
Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine (/vaccines-blood-biologics/approved-products/meningococcal-groups-c-y-and-w-135-polysaccharide-diphtheria-toxoid-conjugate-vaccine)	Menactra
Meningococcal Group B Vaccine (/vaccines-blood-biologics/approved-products/bexsero)	BEXSERO
Meningococcal Group B Vaccine (/vaccines-blood-biologics/approved-products/trumenba)	TRUMENBA
Meningococcal Polysaccharide Vaccine, Groups A, C, Y and W-135 Combined (/vaccines-blood-biologics/approved-products/meningococcal-polysaccharide-vaccine-groups-c-y-and-w-135-combined)	Menomune-A/C/Y/W-135
Meningococcal (Groups A, C, Y, W) Conjugate Vaccine (/vaccines-blood-biologics/menquadfi)	MenQuadfi
Plague Vaccine	No trade name
Pneumococcal Vaccine, Polyvalent (/vaccines-blood-biologics/approved-products/pneumococcal-vaccine-polyvalent)	Pneumovax 23
Pneumococcal 13-valent Conjugate Vaccine (/vaccines-blood-biologics/approved-products/pneumococcal-13-valent-conjugate-vaccine-diphtheria-crm197-protein) (Diphtheria CRM_{197} Protein)	Prevnar 13

Poliovirus Vaccine Inactivated (Human Diploid Cell)	Poliovax
Poliovirus Vaccine Inactivated (Monkey Kidney Cell) (/vaccines-blood-biologics/approved-products/poliovirus-vaccine-inactivated-monkey-kidney-cell)	IPOL
Rabies Vaccine (/vaccines-blood-biologics/approved-products/rabies-vaccine)	Imovax
Rabies Vaccine (/vaccines-blood-biologics/approved-products/rabavert-rabies-vaccine)	RabAvert
Rabies Vaccine Adsorbed	No Trade Name
Rotavirus Vaccine, Live, Oral (/vaccines-blood-biologics/approved-products/rotavirus-vaccine-live-oral)	ROTARIX
Rotavirus Vaccine, Live, Oral, Pentavalent (/vaccines-blood-biologics/approved-products/rotateq)	RotaTeq
Smallpox and Monkeypox Vaccine, Live, Non-Replicating (/vaccines-blood-biologics/jynneos)	JYNNEOS
Smallpox (Vaccinia) Vaccine, Live (/vaccines-blood-biologics/approved-products/smallpox-vaccinia-vaccine-live)	ACAM2000
Tetanus & Diphtheria Toxoids, Adsorbed (/vaccines-blood-biologics/approved-products/tetanus-diphtheria-toxoids-adsorbed)	TDVAX
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use (/vaccines-blood-biologics/approved-products/tenivac)	TENIVAC
Tetanus Toxoid Adsorbed (/vaccines-blood-biologics/approved-products/diphtheria-and-tetanus-toxoids-adsorbed)	No Trade Name
Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed (/vaccines-blood-biologics/approved-products/tetanus-toxoid-reduced-diphtheria-toxoid-and-acellular-pertussis-vaccine-adsorbed)	Adacel

Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed (/vaccines-blood-biologics/approved-products/tetanus-toxoid-reduced-diphtheria-toxoid-and-acellular-pertussis-vaccine-adsorbed)	Boostrix
Typhoid Vaccine Live Oral Ty21a (/vaccines-blood-biologics/approved-products/vivotif)	Vivotif
Typhoid Vi Polysaccharide Vaccine (/vaccines-blood-biologics/approved-products/typhim-vi)	TYPHIM Vi
Varicella Virus Vaccine Live (/vaccines-blood-biologics/approved-products/varivax)	Varivax
Yellow Fever Vaccine (/vaccines-blood-biologics/approved-products/yellow-fever-vaccine)	YF-Vax
Zoster Vaccine, Live, (Oka/Merck) (/vaccines-blood-biologics/approved-products/zoster-vaccine-live)	Zostavax
Zoster Vaccine Recombinant, Adjuvanted (/vaccines-blood-biologics/vaccines/shingrix)	SHINGRIX

EXHIBIT 356

TOXICOLOGICAL PROFILE FOR ALUMINUM

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

September 2008

UPDATE STATEMENT

A Toxicological Profile for Aluminum, Draft for Public Comment, was released in September 2006. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

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1.2 WHAT HAPPENS TO ALUMINUM WHEN IT ENTERS THE ENVIRONMENT?

Sources	Aluminum occurs naturally in soil, water, and air. High levels in the environment can be caused by the mining and processing of aluminum ores or the production of aluminum metal, alloys, and compounds. Small amounts of aluminum are released into the environment from coal-fired power plants and incinerators.
Break down • **Air** • **Water and soil**	Aluminum cannot be destroyed in the environment. It can only change its form or become attached or separated from particles. Aluminum particles in air settle to the ground or are washed out of the air by rain. However, very small aluminum particles can stay in the air for many days. Most aluminum-containing compounds do not dissolve to a large extent in water unless the water is acidic or very alkaline.

For more information on aluminum in the environment, see Chapter 6.

1.3 HOW MIGHT I BE EXPOSED TO ALUMINUM?

Food—primary source of exposure	Unprocessed foods like fresh fruits, vegetables, and meat contain very little aluminum. Aluminum compounds may be added during processing of foods, such as: • flour • baking powder • coloring agents • anticaking agents An average adult in the United States eats about 7–9 mg of aluminum per day in their food.
Air	Most people take in very little aluminum from breathing. Levels of aluminum in the air generally range from 0.005 to 0.18 micrograms per cubic meter ($\mu g/m^3$), depending on location, weather conditions, and type and level of industrial activity in the area. Most of the aluminum in the air is in the form of small suspended particles of soil (dust). Aluminum levels in urban and industrial areas may be higher and can range from 0.4 to 8.0 $\mu g/m^3$.

the only neurological end points examined were brain weight and histology of the brain; no function tests were performed.

There is limited information on aluminum toxicity following dermal exposure. Application of aluminum compounds to the skin, such as aluminum chloride in ethanol or alum, may cause rashes in some people. Skin damage has been observed in mice, rabbits, and pigs exposed to aluminum chloride or aluminum nitrate, but not following exposure to aluminum sulfate, aluminum hydroxide, aluminum acetate, or aluminum chlorhydrate.

There is a fair amount of human data on the toxicity of aluminum following oral exposure. However, the preponderance of human studies are in patients with reduced renal function who accumulated aluminum as a result of long-term intravenous hemodialysis therapy with aluminum-contaminated dialysis fluid and, in many cases, concurrent administration of high oral doses of aluminum to regulate phosphate levels (i.e., reduce uptake of phosphate by binding it in the gut) and have limited usefulness in predicting toxicity in the general population because the very large aluminum exposure levels and impaired renal function results in aluminum accumulation. Dialysis encephalopathy syndrome (also referred to as dialysis dementia) can result from this accumulation of aluminum in the brain. Dialysis encephalopathy is a degenerative neurological syndrome, characterized by the gradual loss of motor, speech, and cognitive functions. Another neurological effect that has been proposed to be associated with aluminum exposure is Alzheimer's disease. Although a possible association was proposed over 40 years ago, this association is still highly controversial and there is little consensus regarding current evidence. A number of studies have found weak associations between living in areas with elevated aluminum levels in drinking water and an increased risk (or prevalence) of Alzheimer's disease; other studies have not found significant associations. In contrast, no significant associations have been found between tea consumption or antacid use and the risk of Alzheimer's disease; although the levels of aluminum in tea and antacids are very high compared to drinking water, aluminum from these sources is poorly absorbed. The available data do not suggest that aluminum is a causative agent of Alzheimer's disease; however, it is possible that it may play a role in the disease development.

Aluminum is found in several ingested over-the-counter products such as antacids and buffered aspirin; clinical studies on health effects of aluminum medicinals in people with normal renal function have been identified. These aluminum-containing products are assumed to be safe in healthy individuals at recommended doses based on historical use. The assumed safety of aluminum is also partly due to the generally regarded as safe (GRAS) status of aluminum-containing food additives. However, there is

some indication that adverse effects can result from long-term use of aluminum-containing medications in some healthy individuals. There are a number of case reports of skeletal changes (e.g., osteomalacia) in adults and children with normal kidney function due to long-term antacid use for the treatment of gastrointestinal disorders. These skeletal effects are secondary to hypophosphatemia and phosphate depletion caused by aluminum impairing phosphorus absorption by binding with dietary phosphorus.

There is a rather extensive database on the oral toxicity of aluminum in animals. These studies clearly identify the nervous system as the most sensitive target of aluminum toxicity and most of the animal studies have focused on neurotoxicity and neurodevelopmental toxicity. Other adverse effects that have been observed in animals orally exposed to aluminum include impaired erythropoiesis in rats exposed to 230 mg Al/kg/day and higher, erythrocyte damage (as evidenced by decreases in hemoglobin, hematocrit, and erythrocyte osmotic fragility, and altered erythrocyte morphology) in rats exposed to 230 mg Al/kg/day and higher, increased susceptibility to infection in mouse dams exposed to 155 mg Al/kg/day, delays in pup maturation following exposure of rats to 53 mg Al/kg/day, and decreases in pup body weight gain in rats and mice exposed to 103 mg Al/kg/day and higher.

Neurodegenerative changes in the brain, manifested as intraneuronal hyperphosphorylated neuro-filamentous aggregates, is a characteristic response to aluminum in certain species and nonnatural exposure situations generally involving direct application to brain tissue, particularly intracerebral and intracisternal administration and *in vitro* incubation in rabbits, cats, ferrets, and nonhuman primates. Oral studies in rats and mice have not found significant histopathological changes in the brain under typical exposure conditions; however, altered myelination was found in the spinal cord of mouse pups exposed to 330 mg Al/kg/day on gestation day 1 through postnatal day 35. Overt signs of neurotoxicity are rarely reported at the doses tested in the available animal studies ($\leq$330 mg Al/kg/day for bioavailable aluminum compounds); rather, exposure to these doses is associated with subtle neurological effects detected with neurobehavioral performance tests. Significant alterations in motor function, sensory function, and cognitive function have been detected following exposure to adult or weanling rats and mice or following gestation and/or lactation exposure of rats and mice to aluminum lactate, aluminum nitrate, and aluminum chloride. The most consistently affected performance tests were forelimb and/or hindlimb grip strength, spontaneous motor activity, thermal sensitivity, and startle responsiveness. Significant impairments in cognitive function have been observed in some studies, although this has not been found in other studies even at higher doses. Adverse neurological effects have been observed in rats and mice at doses of 100–200 mg Al/kg/day and neurodevelopmental effects have been observed in rats and mice at doses of 103–330 mg Al/kg/day.

A number of human studies have examined the occurrence of cancer among aluminum industry workers and found a higher-than-expected cancer mortality rate, but this is probably due to the other potent carcinogens to which they are exposed, such as polycyclic aromatic hydrocarbons (PAHs) and tobacco smoke. Available cancer studies in animals have not found biologically relevant increases in malignant tumors. The International Agency for Research on Cancer (IARC) concluded that aluminum production was carcinogenic to humans and that pitch volatiles have fairly consistently been suggested in epidemiological studies as being possible causative agents. The Department of Health and Human Services and EPA have not evaluated the human carcinogenic potential of aluminum.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for aluminum. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

No acute-, intermediate-, or chronic-duration inhalation MRLs were derived for aluminum. Results from human and animal studies suggest that the respiratory tract, particularly the lung, is a sensitive target of airborne aluminum toxicity; human studies also suggest that the nervous system may also be a target of

inhaled aluminum. Interpretation of the human data is complicated by the lack of exposure assessment and the potential for concomitant exposure to other toxic compounds. Numerous studies have found impaired lung function in a variety of aluminum workers (Abbate et al. 2003; Al-Masalkhi and Walton 1994; Bast-Pettersen et al. 1994; Bost and Newman 1993; Burge et al. 2000; Chan-Yeung et al. 1983; Herbert et al. 1982; Hull and Abraham 2002; Jederlinic et al. 1990; Korogiannos et al. 1998; Miller et al. 1984b; Radon et al. 1999; Simonsson et al. 1985; Vandenplas et al. 1998). Other effects that have been observed include occupational asthma (Abramson et al. 1989; Burge et al. 2000; Kilburn 1998; Vandenplas et al. 1998) and pulmonary fibrosis (Al-Masalkhi and Walton 1994; De Vuyst et al. 1986; Edling 1961; Gaffuri et al. 1985; Gilks and Churg 1987; Jederlinic et al. 1990; Jephcott 1948; McLaughlin et al. 1962; Mitchell et al. 1961; Musk et al. 1980; Riddell 1948; Shaver 1948; Shaver and Riddell 1947; Ueda et al. 1958; Vallyathan et al. 1982).

Acute-, intermediate-, and chronic-duration animal studies have also reported respiratory effects. These respiratory effects include increases in alveolar macrophages, granulomatous lesions in the lungs and peribronchial lymph nodes, and increases in lung weight (Drew et al. 1974; Klosterkotter 1960; Pigott et al. 1981; Steinhagen et al. 1978; Stone et al. 1979). The lung effects observed in humans and animals are suggestive of dust overload. Dust overload occurs when the volume of dust in the lungs markedly impairs pulmonary clearance mechanisms. Lung overload is not dependent on the inherent toxicity of the compound, and dust overloading has been shown to modify both the dosimetry and toxicological effects of the compound (Morrow 1988). When excessive amounts of widely considered benign dusts are persistently retained in the lungs, the resultant lung effects are similar to those observed following exposure to dusts that are highly toxic to the lungs. Because it is unclear whether the observed respiratory effects are related to aluminum toxicity or to dust overload, inhalation MRLs based on respiratory effects were not derived.

Subtle neurological effects have also been observed in workers chronically exposed to aluminum dust or fumes. These effects include impaired performance on neurobehavioral tests (Akila et al. 1999; Bast-Pettersen et al. 2000; Buchta et al. 2003, 2005; Hänninen et al. 1994; Hosovski et al. 1990; Polizzi et al. 2001; Rifat et al. 1990; Riihimäki et al. 2000; Sjögren et al. 1990) and increased reporting of subjective neurological symptoms (Bast-Pettersen et al. 1994, 2000; Hänninen et al. 1994; Hosovski et al. 1990; Iregren et al. 2001; Rifat et al. 1990; Riihimäki et al. 2000; Sim et al. 1997; Sjögren et al. 1990, 1996; White et al. 1992). Neurological exams in the available animal studies (Steinhagen et al. 1978; Stone et al. 1979) have been limited to measurement of brain weight and/or histopathology of the brain; no function tests were performed. The identification of neurotoxicity as a sensitive end point in workers

exposed to aluminum dust and fumes is well supported by a large number of animal studies reporting a variety of neurobehavioral alterations following oral exposure. However, the poor characterization of aluminum exposure in the occupational exposure studies precludes using these studies to develop an inhalation MRL for aluminum.

Oral MRLs

Data on health effects of ingested aluminum in humans are unsuitable for MRL consideration because studies have centered on specific patient populations (i.e., dialysis, neurodegenerative disease) and are not the types typically used in risk evaluation. Studies in patients with reduced renal function who accumulated aluminum as a result of long-term intravenous hemodialysis therapy with aluminum-contaminated dialysate and the use of aluminum-containing phosphate binding agents provide evidence that aluminum is an important etiologic factor in dialysis-related health disorders, particularly the neurological syndrome dialysis encephalopathy. The effects are manifested under unnatural exposure conditions in which the gastrointestinal barrier is bypassed (exposure to aluminum in dialysate fluid) and aluminum excretion is impaired by the poor renal function. There are case reports of skeletal changes (e.g., osteomalacia) consequent to long-term ingestion of antacids in healthy adults and children with normal kidney function (Carmichael et al. 1984; Chines and Pacifici 1990; Pivnick et al. 1995; Woodson 1998), but these effects are attributable to an interaction between aluminum and phosphate in the gut (aluminum binds with phosphate in the gut resulting in decreased phosphate absorption and hypophosphatemia). Although the use of aluminum medicinals in people is widespread, there are a limited number of experimental studies that examined the potential toxicity of the aluminum in these medicinals in individuals with normal renal function.

Derivation of an MRL(s) for aluminum based on animal studies is complicated by limitations in the database, particularly the lack of information on aluminum content in the base diet. As discussed in the introduction to Section 3.2.2, commercial laboratory animal feeds contain high levels of aluminum that can significantly contribute to total experimental exposure. Due to the likelihood of significant base dietary exposure to aluminum, studies with insufficient information on aluminum content in the base diet must be assumed to underestimate the actual aluminum intake. The magnitude of the underestimate can be considerable; for example, approximate feed concentrations of 250 and 350 ppm aluminum reported in some rat and mouse studies, respectively (Colomina et al. 1998; Domingo et al. 1993; Oteiza et al. 1993), are roughly equivalent to daily doses of 25 mg Al/kg/day (rats) and 68 mg Al/kg/day (mice), which represents a significant portion of the lethal dose for these species. Consequently, although studies with

inadequate data on base dietary levels of aluminum provide useful information on health effects of aluminum, no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) from these studies cannot be assumed to be accurate, are not suitable for comparing with effect levels from studies that used diets with known amounts of aluminum, and are inappropriate for MRL consideration.

The available data were considered inadequate for derivation of an acute-duration oral MRL for aluminum. Two studies were identified that provided sufficient information on the levels of aluminum in the basal diet. McCormack et al. (1979) and Domingo et al. (1989) did not find any significant alterations in pup viability/lethality, pup body weight, or the incidence of malformation in rats exposed to 110 mg Al/kg/day as aluminum chloride in the diet on gestation days 6–19 (McCormack et al. 1979) or 141 mg Al/kg/day as aluminum nitrate administered via gavage on gestation days 6–15 (Domingo et al. 1989). Neither study evaluated the potential neurotoxicity of aluminum following acute-duration exposure; intermediate-duration studies provide strong evidence that the nervous system (in adults and developing organisms) is the most sensitive target of aluminum toxicity.

- An MRL of 1 mg Al/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to aluminum.

A fair number of animal studies have examined the oral toxicity of aluminum following intermediate-duration exposure. A subset of these studies that provide information on the aluminum content of the basal diet and involved exposure to aluminum via the diet or drinking water will be the focus of this discussion. With the possible exception of reproductive function, these studies have examined most potential end points of aluminum toxicity. Systemic toxicity studies have not consistently reported adverse effects in rats exposed to up to 284 mg Al/kg/day (Domingo et al. 1987b; Gomez et al. 1986; Konishi et al. 1996), mice exposed to doses as high as 195 mg Al/kg/day (Oteiza et al. 1989), or dogs exposed to doses as high as 88 mg Al/kg/day (Katz et al. 1984; Pettersen et al. 1990). An increased susceptibility to bacterial infections was observed in mouse dams exposed to 155 mg Al/kg/day as aluminum lactate in the diet on gestation day 1 through lactation day 21 (Yoshida et al. 1989). However, a similar aluminum dose did not result in a change in susceptibility in virgin female mice exposed to 107 mg Al/kg/day as aluminum lactate in the diet for 6 weeks (Yoshida et al. 1989). Immunological alterations (decreased spleen concentrations of interleukin-2, interferon g, and tumor necrosis factor and a decrease in CD^{4+} cells) were observed in mice exposed to 200 mg Al/kg/day as aluminum lactate in the diet on gestation day 1 through postnatal day 180 (Golub et al. 1993). There is limited information on the potential for aluminum to induce reproductive effects. Although a number of studies have reported no

alterations in the occurrence of resorption, litter size, sex ratio, or pup body weight, no studies have examined fertility or potential effects on sperm morphology or motility. A significant alteration in gestation length was observed in mice exposed to 155 or 330 mg Al/kg/day as aluminum lactate in the diet on gestation day 1 through lactation 21 (Donald et al. 1989); in the aluminum exposed mice, 4 of the 17 litters were born earlier or later (days 17, 19, or 20 versus day 18 in controls) than control litters. However, this has not been reported in other studies in mice or rats (Colomina et al. 2005; Golub and Germann 2001; Golub et al. 1992a, 1995).

The preponderance of available intermediate-duration studies has focused on the potential for aluminum to induce neurological and neurodevelopmental effects. Although neurotoxicity of aluminum has not been established in people with normal renal function, the data for dialysis encephalopathy (as well as some occupational studies) establish that the human nervous system is susceptible to aluminum and neurotoxicity is a well-documented effect of aluminum in orally-exposed in mice and rats. A wide variety of behavioral tests were conducted in rats and mice, in which the most consistently affected behaviors involve motor function. Alterations in forelimb and hindlimb grip strength have been observed in adult mice exposed to 195 mg Al/kg/day as aluminum lactate in the diet for 90 days (Golub et al. 1992b), mice (6 weeks of age at study beginning) exposed to 195 mg Al/kg/day as aluminum lactate in the diet for 5–7 weeks (Oteiza et al. 1993), the offspring of mice exposed on gestation day 1 through lactation day 21 to 155 mg Al/kg/day (Donald et al. 1989; Golub et al. 1995) or 250 mg Al/kg/day (Golub et al. 1995) as aluminum lactate, and the offspring of rats exposed to 103 mg Al/kg/day as aluminum nitrate in drinking water (with added citric acid) for 15 days prior to mating and on gestation day 1 through lactation day 21 (Colomina et al. 2005). Decreases in spontaneous motor activity were observed in mice exposed to 130 mg Al/kg/day for 6 weeks (Golub et al. 1989) or 195 mg Al/kg/day for 90 days (Golub et al. 1992b). Motor impairments have also been detected in mice in the wire suspension test in which offspring exposed to 130 mg Al/kg/day had a shorter latency to fall from the wire and in the rotorod test in which offspring exposed to 260 mg Al/kg/day had a higher number of rotations (which occur when the animals lost its footing, clung to the rod, and rotated with it for a full turn) (Golub and Germann 2001). Neurobehavioral alterations that have occurred at similar dose levels include decreased responsiveness to auditory or air-puff startle (Golub et al. 1992b, 1995), decreased thermal sensitivity (Golub et al. 1992a), increased negative geotaxis latency (Golub et al. 1992a), and increased foot splay (Donald et al. 1989). Additionally, one study found significant impairment in performance of the water maze test in offspring of mice exposed to 130 mg Al/kg/day on gestation day 1 through lactation day 21 (Golub and Germann 2001). Colomina et al. (2005) did not find alterations in this test in rats exposed to 53 mg Al/kg/day; however, this study did not run probe tests, which showed significant

alterations in the Golub and Germann (2001) study. Other studies have utilized passive avoidance tests or operant training tests to evaluate potential impairment of cognitive function. However, the interpretation of the results of these tests is complicated by an increase in food motivation in aluminum exposed mice (Golub and Germann 1998).

There is also strong evidence that gestational and/or lactational exposure can cause other developmental effects. Gestation and/or lactation exposure can result in significant decreases in pup body weight gain in rats and mice (Colomina et al. 2005; Golub and Germann 2001; Golub et al. 1992a). The decreases in pup body weight are often associated with decreases in maternal body weight during the lactation phase of the study; however, decreases in body weight have also been observed in a cross-fostering study when gestation-exposed pups were nursed by control mice (Golub et al. 1992a). Other studies involving gestation and lactation exposure to aluminum did not find changes in pup growth in mice (Donald et al. 1989; Golub and Germann 1998; Golub et al. 1995). In rats, a delay in physical maturation, particularly delays in vagina opening, testes descent, and incisor eruption, has been reported at 53 mg Al/kg/day (Colomina et al. 2005). In the Colomina et al. (2005) study, a delay in vagina opening was observed in rat offspring exposed to 53 mg Al/kg/day. The number of days to vagina opening was 31.1, 40.9, and 45.9 days in the control, 53, and 103 mg Al/kg/day groups, respectively. Delays in maturations were also observed for testes descent (23.9, 22.8, and 27.1 days in the control, 53, and 103 mg Al/kg/day groups, significant at 103 mg Al/kg/day) and incisor eruption in males (5.5, 6.1, and 5.3 days, significant at 53 mg Al/kg/day, but not at 103 mg Al/kg/day). Significant delays in vagina opening and testes descent were also observed at 103 mg Al/kg/day in the offspring of rats similarly exposed but with the addition of restraint stress on gestation days 6–20. The mean number of days to maturation in the control, 53, and 103 mg Al/kg/day groups were 32.5, 40.4, and 44.9 days for vagina opening and 24.9, 23.2, and 27.7 days for testes descent. However, another study by Colomina et al. (1999) did not find significant delays in vagina opening or testes descent, but did find significant delays in pinna attachment and eye opening following administration of 75 mg/kg/day (15 mg Al/kg/day) aluminum chloride via intraperitoneal injection to mice on gestation days 6–15. Another study did not find delays in pinna attachment, eye opening, or incisor eruption in the offspring of rats administered via gavage 73 mg Al/kg/day as aluminum chloride (aluminum content of the diet was not reported) on gestation days 8–20 (Misawa and Shigeta 1992). Collectively, these studies provide equivocal evidence that aluminum induces delays in maturation.

The Golub et al. (1989), Golub and Germann (2001), and Colomina et al. (2005) studies identified the lowest LOAELs for the critical effects (neurotoxicity, neurodevelopmental toxicity, and delays in

maturation) and were considered as possible principal studies. Golub et al. (1989) identified the lowest LOAEL for neurotoxicity. In this study in which mice were exposed to aluminum lactate in the diet for 6 weeks, significant decreases in total activity and vertical activity (rearing) were observed at 130 mg Al/kg/day; no significant alterations were observed at 62 mg Al/kg/day. One limitation of this study is that motor activity was the only neurobehavioral test evaluated; other studies have shown that grip strength is one of the more sensitive end points. Golub and Germann (2001) examined a number of sensitive end points of neurodevelopmental toxicity in the offspring of mice exposed to aluminum lactate in the diet on gestation day 1 through lactation day 21, after which the pups were fed a diet containing the same levels of aluminum as the dams on postnatal days 21–35. The study identified a NOAEL of 26 mg Al/kg/day and a LOAEL of 130 mg Al/kg/day for alterations in tests of motor function (a shorter latency to fall off a wire) and cognitive function (impaired performance in the water maze test). This study used a suboptimal diet, which complicates the interpretation of the study results. The dietary levels of phosphorus, calcium, magnesium, iron, and zinc were lower than the National Research Council's recommendation in an attempt to mimic the intakes of these nutrients by young women. The investigators noted that even though the intakes of several nutrients were below the recommendations, the diet was not deficient. The impact of the suboptimal diet on the developmental toxicity of aluminum is not known. The observed effects are similar to those reported in other studies, as are the adverse effect levels. In the Colomina et al. (2005) study, a significant decrease in forelimb grip strength was observed in the offspring of rats exposed to 103 mg Al/kg/day as aluminum nitrate in the drinking water (with citric acid added to increase aluminum absorption) for 15 days prior to mating and during gestation and lactation; grip strength was not adversely affected at 53 mg Al/kg/day. This study also found significant delays in vagina opening at 53 mg Al/kg/day. As previously noted, there are limited data to confirm or refute the identification of delays in maturation as a critical effect of aluminum. The delays in maturation may be secondary to decreases in maternal weight or food intake or decreases in pup body weight and/or food intake; however, these data are only reported for some time periods. The Golub et al. (1989) study was not selected as the principal study because the NOAEL of 62 mg Al/kg/day identified in this study is higher than the dose associated with delayed maturation in the Colomina et al. (2005) study. The Golub and Germann (2001) and Colomina et al. (2005) studies were selected as co-principal studies. A short description of these studies follows.

In the Golub and Germann (2001) study, groups of pregnant Swiss Webster mice were exposed to 0, 100, 500, or 1,000 mg Al/kg diet on gestational days 0–21 and during lactation until day 21. On postnatal day (PND) 21, one male and one female pup from each litter were placed on the same diet as the dam. The offspring were exposed until PND 35. The composition of the diet was modified from the National

Research Council's recommendations; the investigators noted that the nutrients were reduced to correspond to the usual intake of these nutrients by young women. The average daily intakes of phosphorus, calcium, magnesium, iron, and zinc in women aged 18–24 years are 83, 56, 71, 69, and 67% of the recommended dietary allowance (RDA); these percents were used to modify the recommended dietary intake for the mice used in this study. Doses of 26, 130, and 260 mg Al/kg/day are calculated by averaging reported estimated doses of 10, 50, and 100 mg Al/kg/day for adults (i.e., at beginning of pregnancy) and 42, 210, and 420 mg Al/kg/day maximal intake during lactation. The doses at lactation were calculated using doses estimated in previous studies with similar exposure protocols performed by the same group of investigators (Golub et al. 1995). At 3 months of age, the females were tested for neurotoxicity using the Morris water maze. At 5 months of age, males were tested for motor activity and function using rotarod, grip strength, wire suspension, mesh pole descent, and beam traversal tests. No alterations in pregnancy weight gain or pup birth weights were observed. At PND 21, significant decreases in pup body weights were observed at 130 and 260 mg Al/kg/day. No information on maternal weight gain during lactation was reported; however, the investigators noted that the decrease in pup weight was not associated with reduced maternal food intake. At PND 35, the decrease in body weight was statistically significant at 260 mg Al/kg/day. On PND 90, female mice in the 260 mg Al/kg/day group weighed 15% less than controls. Decreases in heart and kidney weights were observed at 260 mg Al/kg/day in the females. Also, increases in absolute brain weight were observed in females at 26 mg Al/kg/day and relative brain weights were observed at 26 or 260 mg Al/kg/day, but not at 130 mg Al/kg/day. In the males, significant decreases in body weight were observed at 130 (10%) and 260 (18%) mg Al/kg/day at 5 months; an increase in food intake was also observed at these doses. In the Morris maze (tested at 3 months in females), fewer animals in the 260 mg Al/kg/day group had escape latencies of <60 seconds during sessions 1–3 (learning phase) and a relocation of the visible cues resulted in increased latencies at 130 and 260 mg Al/kg/day. Body weight did not correlate with latency to find the platform or with the distribution of quadrant times. The investigators concluded that controls used salient and/or nonsalient cues, 26 and 130 mg Al/kg/day animals used both cues, but had difficulty using only one cue, and 260 mg Al/kg/day animals only used the salient cues. In the males tested at 5 months, a significant decrease in hindlimb grip strength was observed at 260 mg Al/kg/day, an increase in the number of rotations on the rotorod as observed at 260 mg Al/kg/day, and a shorter latency to fall in the wire suspension test was observed at 130 and 260 mg Al/kg/day. The investigators noted that there were significant correlations between body weight and grip strength and number of rotations. When hindlimb grip strength was statistically adjusted for body weight, the aluminum-exposed mice were no longer significantly different from controls; the number of rotations was still significantly different from control after adjustment for body weight.

In the Colomina et al. (2005) study, groups of female Sprague Dawley rats were exposed to 0, 50, or 100 mg Al/kg/day aluminum nitrate nonahydrate in drinking water; citric acid (710, 355, and 710 mg/kg/day in the control, 50, and 100 ppm groups, respectively) was added to the drinking water to increase aluminum absorption. The adult rats were exposed to aluminum for 15 days prior to mating and during gestation and lactation periods; after weaning, the pups were exposed to the same aluminum concentration as the mothers from PND 21 through 68. The basal diet (Panlab rodent chow) contained 41.85 µg Al/g diet. Aluminum doses were calculated by adding the basal dietary aluminum doses (calculated using reference values for mature Sprague-Dawley rats) to reported aluminum doses from water; the total aluminum doses were 3, 53, and 103 mg Al/kg/day. In addition to aluminum exposure, some animals in each group underwent restraint stress for 2 hours/day on gestation days 6–20; the restraint consisted of placing the rats in cylindrical holders. The following neurobehavioral tests were performed on the offspring: righting reflex (PNDs 4, 5, 6), negative geotaxis (PNDs 7, 8, 9), forelimb grip strength (PNDs 10–13), open field activity (PND 30), passive avoidance (PND 35), and water maze (only tested at 53 mg/kg/day on PND 60). The rats were killed on PND 68. No significant alterations in body weight, food consumption, or water consumption were observed during gestation in the dams exposed to aluminum. The investigators noted that decreases in water and food consumption were observed during the lactation period in the rats exposed to 103 mg Al/kg/day, but the data were not shown and maternal body weight during lactation was not mentioned. No significant alterations in the number of litters, number of fetuses per litter, viability index, or lactation index were observed. Additionally, no differences in days at pinna detachment or eye opening were observed. Age at incisor eruption was significantly higher in males exposed to 53 mg/kg/day, but not in males exposed to 103 mg/kg/day or in females. A significant delay in age at testes descent was observed at 103 mg/kg/day and vagina opening was delayed at 53 and 103 mg/kg/day. A decrease in forelimb grip strength was observed at 103 mg/kg/day; no alterations in other neuromotor tests were observed. Additionally, no alterations in open field behavior or passive avoidance test were observed. In the water maze test, latency to find the hidden platform was decreased in the 53 mg/kg/day group on test day 2, but not on days 1 or 3; no significant alteration in time in the target quadrant was found.

The Golub and Germann (2001) and Colomina et al. (2005) studies identify four end points that could be used as the point of departure for derivation of the intermediate-duration oral MRL:

(1) latency to fall off wire in wire suspension test; adverse effect level of 130 mg Al/kg/day, no effect level of 26 mg Al/kg/day (Golub and Germann 2001);

(2) latency to locate the platform following cue relocation in the water maze test; adverse effect level of 130 mg Al/kg/day, no effect level of 26 mg Al/kg/day (Golub and Germann 2001);

(3) decreased forelimb grip strength; adverse effect level of 103 mg Al/kg/day, no effect level of 53 mg Al/kg/day (Colomina et al. 2005); and

(4) delay in vagina opening; adverse effect level of 53 mg Al/kg/day, no effect level not identified (Colomina et al. 2005).

Benchmark dose (BMD) modeling was considered for each of these end points. As discussed in Appendix A, BMD modeling was not used to identify the point of departure due to incomplete reporting of the data or because the models did not provide adequate fit.

Using a NOAEL/LOAEL approach, the NOAEL of 26 mg Al/kg/day identified in the Golub and Germann (2001) study was selected as the point of departure for the MRL. An MRL based on this NOAEL should be protective for neurological effects, neurodevelopmental effects, and for delays in maturation. Dividing the NOAEL by an uncertainty factor of 100 (10 to account for the extrapolation from mice to humans and 10 for human variability) and a modifying factor of 0.3 to account for possible differences in the bioavailability of the aluminum lactate used in the Golub and Germann (2001) study and the bioavailability of aluminum from drinking water and a typical U.S. diet results in an MRL of 1 mg Al/kg/day. No studies were identified that estimated the bioavailability of aluminum lactate following long-term dietary exposure; however, a bioavailability of 0.63% was estimated in rabbits receiving a single dose of aluminum lactate (Yokel and McNamara 1988). Yokel and McNamara (2001) and Powell and Thompson (1993) suggest that the bioavailability of aluminum from the typical U.S. diet was 0.1%; the bioavailability of aluminum from drinking water ranges from 0.07 to 0.39% (Hohl et al. 1994; Priest et al. 1998; Stauber et al. 1999; Steinhausen et al. 2004). These data suggest that aluminum lactate has a higher bioavailability than aluminum compounds typically found in drinking water or the diet.

- An MRL of 1 mg Al/kg/day has been derived for chronic-duration oral exposure (365 days or longer) to aluminum.

A small number of animal studies examined the chronic toxicity of aluminum. Schroeder and Mitchener (1975a, 1975b) examined the systemic toxicity of aluminum following lifetime exposure of rats and mice to very low doses of aluminum sulfate in the drinking water. Although the levels of aluminum in the diet were not reported, they are assumed to be low because the animals were fed a low-metal diet in metal-free environmental conditions. Studies conducted by Roig et al. (2006) and Golub et al. (2000) primarily

4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Aluminum is a naturally occurring element that appears in the second row of Group 13 (IIIA) of the periodic table (O'Neil et al. 2001). Table 4-1 lists common synonyms and other pertinent identification information for aluminum and selected aluminum compounds.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Aluminum is a silvery-white, malleable, and ductile metal. In moist air, a protective oxide coating of aluminum oxide is formed on its surface. In compounds, aluminum typically occurs in its +3 oxidation state (Lide 2005; O'Neil et al. 2001). Table 4-2 lists important physical and chemical properties of aluminum and selected aluminum compounds.

5.3 USE

In 2006, transportation accounted for an estimated 40% of domestic consumption of aluminum, predominantly as automotive applications, with the remainder used in packaging, 28%; building, 13%; consumer durables, 7%; electrical, 5%; and other, 7% (USGS 2007c).

Aluminum chloride, anhydrous form, is used as an acid catalyst (especially in Friedel-Crafts-type reactions), as a chemical intermediate for other aluminum compounds, in the cracking of petroleum, in the manufacture of rubbers and lubricants, and as an antiperspirant. The hexahydrate form is used in preserving wood, disinfecting stables and slaughterhouses, in deodorants and antiperspirants, in cosmetics as a topical astringent, in refining crude oil, dyeing fabrics, and manufacturing parchment paper (O'Neil et al. 2001).

Aluminum chlorohydrate is an ingredient in commercial antiperspirant and deodorant preparations and is also used for water purification and treatment of sewage and plant effluent (Lewis 2001)

Aluminum hydroxide (alumina trihydrate) is used as an adsorbent, emulsifier, ion-exchanger, mordant in dyeing, and filtering medium. It is also used in the manufacturing of glass, paper, ceramics and pottery, printing inks, lubricating compositions, detergents, in the waterproofing of fabrics, in antiperspirants, dentifrices, and as a vaccine adjuvant (Baylor et al. 2002; Lewis 2001; O'Neil et al. 2001). Aluminum hydroxide is used as a flame retardant in the interiors of automobiles, commercial upholstered furniture, draperies, wall coverings, and carpets (Subcommittee on Flame-Retardant Chemicals 2000). Aluminum hydroxide is used as an antacid (O'Neil et al. 2001). Finely divided (0.1–0.6 microns) aluminum hydroxide is used for rubber reinforcing agent, paper coating, filler, and cosmetics (Lewis 2001). Aluminum hydroxide is also used pharmaceutically, as an antihyperphosphatemic, to lower the plasma phosphorus levels of patients with renal failure (O'Neil et al. 2001).

Aluminum nitrate is used in textiles (mordant), leather tanning, the manufacturing of incandescent filaments, catalysts in petroleum refining, nucleonics, anticorrosion agent, nitrating agent, and antiperspirants (Lewis 2001; O'Neil et al. 2001).

In 2006, 96% of the bauxite consumed in the United States was refined to alumina (aluminum oxide), with the remaining 4% consumed in nonmetallurgical uses, such as abrasives, chemicals, and refactories. Of the total alumina used in the United States in 2006, approximately 87% was used for primary

aluminum smelters and the remainder was used for nonmetallurgical uses, including abrasives, chemicals, refactories, and in specialty industries (USGS 2007a, 2007d). Other uses of aluminum oxide are in the manufacture of ceramics, electrical insulators, catalyst and catalyst supports, paper, spark plugs, crucibles and laboratory works, adsorbent for gases and water vapors, chromatographic analysis, fluxes, light bulbs, artificial gems, heat resistant fibers, food additive (dispersing agent), and in hollow-fiber membrane units used in water desalination, industrial ultrafiltration, and hemodialysis (HSDB 2007; Lewis 2001). Another application of aluminum oxide, which may have wide occupational use in the future, is as a dosimeter for measuring personnel radiation exposure (McKeever et al. 1995; Radiation Safety Guide 1999; Radiation Safety Newsletter 1998).

Aluminum phosphate is used in ceramics, dental cements, cosmetics, paints and varnishes, pharmaceuticals (antacid), and in paper and pulp industries (Lewis 2001; O'Neil et al. 2001). It is also used as a vaccine adjuvant (Baylor et al. 2002; Malakoff 2000). Aluminum phosphate, as basic sodium aluminum phosphate (SALP), is used as an emulsifying agent in pasteurized processed cheese, cheese food, and cheese spread. Acidic SALP is used as a leavening agent in cereal foods and related products, such as self-rising flour, prepared cake mixes, pancakes, waffles, and refrigerated or frozen dough or batter products (Chung 1992; Saiyed and Yokel 2005).

Aluminum phosphide is a fumigant used primarily for indoor fumigation of raw agricultural commodities, animal feeds, processed food commodities, and non-food commodities in sealed containers or structures to control insects, and for outdoor fumigation of burrows to control rodents and moles in nondomestic areas, noncropland, and agricultural areas. Aluminum phosphide reacts with the moisture in the atmosphere to produce phosphine gas, which is the substance that is active as a pesticide. Based on available pesticide survey usage information for 1987–1996, the estimated annual usage of aluminum phosphide is about 1.6 million pounds active ingredient. Major uses of aluminum phosphide include fumigation of wheat, peanuts, and stored corn. It was noted that usage estimates for aluminum phosphide are not precise due to scarcity of usage data sources for postharvest agriculture and non-agriculture uses/sites. All aluminum phosphide containing products have been classified as restricted use (EPA 1998). According to the National Pesticide Information Retrieval System, there are five active registrants for aluminum phosphide (NPIRS 2008).

Aluminum sulfate (alum) is used in leather tanning, sizing paper, as a mordent in dyeing, water purification, fireproofing and waterproofing of cloth, clarifying oils and fats, treating sewage, waterproofing concrete, deodorizing and decolorizing of petroleum, antiperspirants, and agricultural

pesticide. It is also used as a food additive, a foaming agent in fire foams, and in the manufacturing of aluminum salts (Lewis 2001; O'Neil et al. 2001). Aluminum sulfate, as sodium aluminum sulfate, is a component of household baking powder (Chung 1992). Alum is also used as a vaccine adjuvant (Baylor et al. 2002; Malakoff 2000). Aluminum potassium sulfate (potash alum) is used in dyeing (mordant), paper, matches, paints, tanning agents, waterproofing agents, aluminum salts, food additives, baking powder, water purification, astringent, and cement hardener (Lewis 2001). Aluminum ammonium sulfate (ammonium alum) is used in dyeing (mordant), water and sewage purification, sizing paper, retanning leather, clarifying agent, food additive, the manufacture of lakes and pigments, and fur treatment (Lewis 2001).

Other aluminum compounds that are used as food additives include aluminum silicates (anticaking agents) and aluminum color additives (lakes) (Saiyed and Yokel 2005; Soni et al. 2001).

5.4 DISPOSAL

Production of finished aluminum products by industrial facilities typically results in the generation of very large amounts of solid aluminum hydroxide anodizing residues (Saunders 1988). These aluminum-anodizing residues are currently classified as nonhazardous under the Federal Resource Conservation and Recovery Act (RCRA) regulations. These residues are typically dewatered to reduce the volume of waste prior to being landfilled. However, the heavy metal content of these solid waste residues can be of concern, especially in production processes using two-step anodizing systems that employ solutions containing elevated heavy metal concentrations. For these types of plants, Saunders (1988) has proposed implementation of a caustic-etch recovery system that will limit both the volume of aluminum-anodizing residue and the heavy metal content of the residue. Additional information on regulations and standards for aluminum and aluminum compounds is summarized in Chapter 8.

Approximately 24.7×10^6 and 1.15×10^5 pounds of aluminum (fume or dust) and aluminum oxide (fibrous forms) were reported for on-site disposal and other releases in 2004. On-site disposal or other releases include emissions to the air, discharges to bodies of water, disposal at the facility to land, and disposal in underground injection wells. Approximately 23.7×10^6 and 1.20×10^6 pounds of aluminum (fume or dust) and aluminum oxide (fibrous forms), respectively, were reported for off-site disposal and other releases in 2004. An off-site disposal or other release is a discharge of a toxic chemical to the environment that occurs as a result of a facility's transferring a waste containing a TRI chemical off-site for disposal or

other release (TRI04 2006). The TRI data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list.

In the United States, about 3 million metric tons of aluminum was recovered from purchased scrap in 2006, with 64% of this coming from new (manufacturing) scrap and 36% from old scrap (discarded aluminum products). Aluminum used beverage cans accounted for about 54% of the reported old scrap consumption in 2006. According to the Aluminum Association, Inc., the recycling rate for used aluminum beverage cans in 2004 was 51.6% (USGS 2007b, 2007c).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

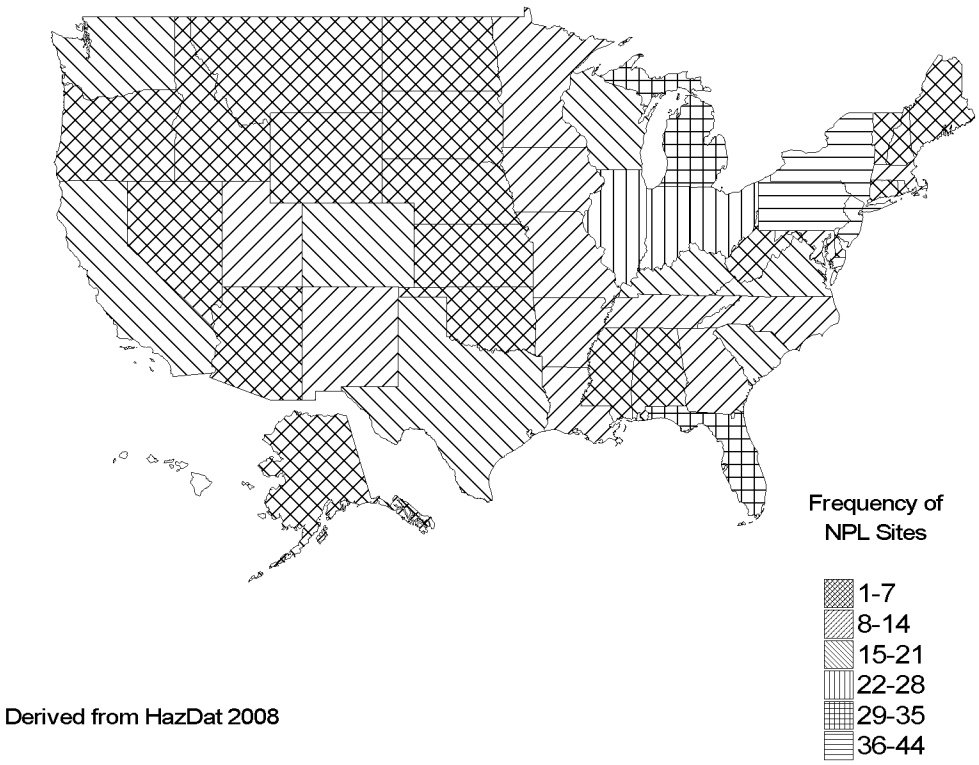
Aluminum has been identified in at least 596 of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2008). However, the number of sites evaluated for aluminum is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 590 are located within the United States, 2 are located in Guam, 3 are located in the Commonwealth of Puerto Rico, and 1 is located in the Virgin Islands (not shown).

Aluminum is the most abundant metal and the third most abundant element in the earth's crust, comprising about 8.8% by weight (88 g/kg). It is never found free in nature and is found in most rocks, particularly igneous rocks as aluminosilicate minerals (Lide 2005; Staley and Haupin 1992). Aluminum is also present in air, water, and many foods. Aluminum enters environmental media naturally through the weathering of rocks and minerals. Anthropogenic releases are in the form of air emissions, waste water effluents, and solid waste primarily associated with industrial processes, such as aluminum production. Because of its prominence as a major constituent of the earth's crust, natural weathering processes far exceed the contribution of releases to air, water, and land associated with human activities (Lantzy and MacKenzie 1979).

The behavior of aluminum in the environment depends upon its coordination chemistry and the characteristics of the local environment, especially pH. The major features of the biogeochemical cycle of aluminum include leaching of aluminum from geochemical formations and soil particulates to aqueous environments, adsorption onto soil or sediment particulates, and wet and dry deposition from the air to land and surface water.

Generally, aluminum is not bioaccumulated to a significant extent. However, certain plants can accumulate high concentrations of aluminum. For example, tea leaves may contain very high concentrations of aluminum, >5,000 mg/kg in old leaves (Dong et al. 1999). Other plants that may contain high levels of aluminum include Lycopodium (Lycopodiaceae), a few ferns, Symplocos (Symplocaceae), and Orites (Proteaceae) (Jansen et al. 2002). Aluminum does not appear to accumulate to any significant degree in cow's milk or beef tissue and is, therefore, not expected to undergo biomagnification in terrestrial food chains (DOE 1984). Similarly, because of its toxicity to many aquatic organisms, including fish, aluminum does not bioconcentrate in aquatic organisms to any significant degree (Rosseland et al. 1990).

Figure 6-1. Frequency of NPL Sites with Aluminum Contamination



Background concentrations of aluminum in rural air typically range from 0.005 to 0.18 μg/m^3 (Hoffman et al. 1969; Pötzl 1970; Sorenson et al. 1974), whereas concentrations in urban and industrial areas can be considerably higher, ranging from 0.4 to 8.0 μg/m^3 (Cooper et al. 1979; Dzubay 1980; Kowalczyk et al. 1982; Lewis and Macias 1980; Moyers et al. 1977; Ondov et al. 1982; Pillay and Thomas 1971; Sorenson et al. 1974; Stevens et al. 1978). Concentrations of aluminum are highly variable in drinking water, ranging from <0.001 to 1.029 mg/L (Schenk et al. 1989). The use of alum (aluminum sulfate) as a flocculent in water treatment facilities typically leads to high aluminum concentrations in finished waters (DOI 1970; Letterman and Driscoll 1988; Miller et al. 1984a). In a survey of 186 community water systems, the median aluminum concentration in finished water receiving coagulation treatment using alum was 0.112 mg/L, compared to 0.043 mg/L in finished water that received no coagulation treatment (Miller et al. 1984a). Dissolved aluminum concentrations in surface and groundwater vary with pH and the humic acid content of the water. High aluminum concentrations in natural water occur only when the pH is <5; therefore, concentrations in most surface water are very low.

Since aluminum is ubiquitous in the environment, the general population will be exposed to aluminum by the inhalation of ambient air and the ingestion of food and water. The consumption of foods containing aluminum-containing food additives are a major sources of aluminum in the diet (Saiyed and Yokel 2005; Soni et al. 2001). The use of other consumer items such as antiperspirants, cosmetics, internal analgesics (buffered aspirins), anti-ulcerative medications, antidiarrheals, and antacids that also contain aluminum compounds will result in exposure to aluminum. The intake of aluminum from food and drinking water is low, especially compared with that consumed by people taking aluminum-containing medicinal preparations. Daily intakes of aluminum from food range from 3.4 to 9 mg/day (Biego et al. 1998; MAFF 1999; Pennington and Schoen 1995), whereas aluminum-containing medications contain much higher levels of aluminum, for example 104–208 mg of aluminum per tablet/capsule/5 mL dose for many antacids (Zhou and Yokel 2005). While aluminum is naturally present in food and water, the greatest contribution to aluminum in food and water by far is the aluminum-containing additives used in water treatment and processing certain types of food such as grain-based products and processed cheese. Aluminum has no known physiological role in the human body (Nayak 2002).

The aluminum content of human breast milk generally ranged from 9.2 to 49 μg/L (Fernandez-Lorenzo et al. 1999; Hawkins et al. 1994; Koo et al. 1988; Simmer et al. 1990; Weintraub et al. 1986). Soy-based infant formulas contain higher concentrations of aluminum, as compared to milk-based infant formulas or breast milk. Recent reports provide average aluminum concentrations of 460–930 μg/L for soy-based

annual urinary aluminum level was 1.4 µmol/L (0.038 mg/L) and the range was 1.08–2.04 µmol/L (0.029–0.055 mg/L) (Valkonen and Aitio 1997). The samples, collected as part of a routine occupational health program, were collected after the weekend as a morning specimen. The mean urinary aluminum concentration in 44 nonexposed persons, who did not use antacid preparations, was 0.33 µmol/L (0.0089 mg/L), and the range and standard deviation were 0.07–0.82 µmol/L (0.002–0.022 mg/L) and 0.18 µmol/L (0.0022 mg/L), respectively. The mean serum aluminum concentration of 21 of these nonexposed individuals was 0.06 µmol/L (0.0016 mg/L), and the range and standard deviation were 0.02–0.13 µmol/L (0.0005–0.0035 mg/L) and 0.03 µmol/L (0.0008 mg/L), respectively. Drablos et al. (1992) studied aluminum concentrations in workers at an aluminum fluoride plant. Mean aluminum concentrations in urine were 0.011 mg/L (range, 0.002–0.046 mg/L) for 15 plant workers, 0.032 mg/L (range, 0.006–0.136 mg/L) for 7 foundry workers, and 0.054 mg/L (range, 0.005–0.492 mg/L) for 12 potroom workers as compared to 0.005 mg/L (range, 0.001–0.037 mg/L) for 230 unexposed controls. Mean aluminum concentrations were 5.06 and 3.74 µg/L in blood, and 6.56 and 6.35 µg/L in urine of 103 workers in the optoelectronic industry and 67 controls, respectively (Liao et al. 2004). Pre- and postshift average aluminum concentrations in urine ranging from 0.13 to 0.153 mg/L were reported in welders from the construction industry (Buchta et al. 2005). Aluminum concentrations in human breast tissue and breast tissue fat of 4–437 nmol/g (0.1–12 µg/g) dry weight and 3–192 nmol/g oil (0.08–5.18 µg/g oil), respectively, have been reported (Exley et al. 2007).

Nieboer et al. (1995) reported background concentrations of aluminum in bone of 1–3 µg/g dry weight. Background aluminum concentrations in brain tissues (primarily grey matter) of healthy individuals typically ranges from 1 to 3 µg/g dry weight or <0.5 µg/g wet weight (Nieboer et al. 1995). Markesbery et al. (1984) determined trace element concentrations in various human brain regions in infants through adults. Aluminum concentrations were shown to increase with increasing age. Mean aluminum concentrations in adults were 0.467 µg/g wet weight, as compared to 0.298 µg/g wet weight in infants. Overall aluminum concentrations ranged from ≤0.050 to 3.05 µg/g, with the highest mean aluminum concentrations in the globus pallius (0.893 µg/g) and the lowest in the superior parietal lobule (0.282 µg/g).

Metal concentrations were determined in spermatozoa and seminal plasma from men working in two industrial companies, a refinery and a polyolefin factory, 40 km east of Helsinki, Finland, and from sperm bank donor candidates from Helsinki, Finland in 1994. Aluminum concentrations in the factory employees were 0.93 and 0.54 mg/kg in spermatozoa and seminal plasma, respectively, and were 2.52 and 0.87 mg/kg in spermatozoa and seminal plasma, respectively, in the donor candidates. The

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comment on the direct final rule. FDA stated that the effective date of the direct final rule would be December 8, 2003, and, if the agency received no significant adverse comments, it would publish a notice of confirmation of the effective date no later than June 11, 2003. FDA received no significant adverse comments within the comment period. Therefore, FDA is confirming that the effective date of the direct final rule is December 8, 2003. As noted in the direct final rule, FDA is publishing this confirmation document 180 days before the effective date to permit affected firms adequate time to take appropriate steps to bring their bottled water products into compliance with the quality standard imposed by the new rule.

Dated: June 2, 2003.

Jeffrey Shuren,

Assistant Commissioner for Policy.

[FR Doc. 03–14477 Filed 6–6–03; 8:45 am]

BILLING CODE 4164–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 310, 350, and 369

[Docket No. 78N–0064]

RIN 0910–AA01

Antiperspirant Drug Products For Over-the-Counter Human Use; Final Monograph

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing a final rule in the form of a final monograph establishing conditions under which over-the-counter (OTC) antiperspirant drug products are generally recognized as safe and effective and not misbranded as part of FDA's ongoing review of OTC drug products. FDA is issuing this final rule after considering public comments on its proposed regulation, issued as a tentative final monograph (TFM), and all new data and information on antiperspirant drug products that have come to the agency's attention.

DATES: *Effective Date*: This rule is effective December 9, 2004.

Compliance Dates: The compliance date for products with annual sales less than $25,000 is June 9, 2005. The compliance date for all other products is December 9, 2004.

FOR FURTHER INFORMATION CONTACT: Gerald M. Rachanow, Center for Drug Evaluation and Research (HFD–560), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301–827–2307.

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I. Background

In the **Federal Register** of October 10, 1978 (43 FR 46694), FDA published an advance notice of proposed rulemaking to establish a monograph for OTC antiperspirant drug products, together with the recommendations of the Advisory Review Panel on OTC Antiperspirant Drug Products (the Panel), which evaluated the data on these products. The agency's proposed regulation (TFM) for OTC antiperspirant drug products was published in the **Federal Register** of August 20, 1982 (47 FR 36492).

In the **Federal Register** of November 7, 1990 (55 FR 46914), the agency issued a final rule establishing that certain active ingredients in OTC drug products are not generally recognized as safe and effective and are misbranded. These ingredients included seven antiperspirant ingredients, which are included in § 310.545(a)(4) (21 CFR 310.545(a)(4)). In this rulemaking, the agency is adding one additional ingredient to this section. (See section III.1 of this document.)

In the **Federal Register** of March 23, 1993 (58 FR 15452), the agency requested public comment on two citizen petitions, and a response to one of the petitions, related to the safety of aluminum compounds in OTC antiperspirant drug products. This final monograph completes the TFM and provides the substantive response to the citizen petitions.

Twenty-four months after the date of publication in the **Federal Register**, for products with annual sales less than $25,000, and 18 months after the date of publication in the **Federal Register**, for all other products, no OTC drug product that is subject to this final rule and that contains a nonmonograph condition may be initially introduced or initially delivered for introduction into interstate commerce unless it is the subject of an approved new drug application (NDA) or abbreviated new drug application. Further, any OTC drug product subject to this final monograph that is repackaged or relabeled after the compliance dates of the final rule must be in compliance with the monograph regardless of the date the product was initially introduced or initially delivered for introduction into interstate commerce. Manufacturers are encouraged to comply voluntarily as soon as possible.

In response to the TFM on OTC antiperspirant drug products and the request for comment on the citizen petitions, the agency received 20 comments. One manufacturer requested an oral hearing before the Commissioner of Food and Drugs on six different issues. Copies of the information considered by the Panel, the comments, and the hearing request are on public display in the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. ''OTC Volumes'' cited in this document refer to information on public display.

The agency received some ''feedback'' communications under the OTC drug review procedures (see the **Federal Registers** of September 29, 1981 (46 FR 47740) and April 1, 1983 (48 FR 14050)). The agency has included these communications in the administrative record and addressed them in this document.

The safety issues raised by the citizen petitions are discussed in section II.F of this document. The agency believes it has adequately responded to the six issues related to the hearing request; therefore, a hearing is not necessary.

II. The Agency's Conclusions on the Comments

A. General Comments on OTC Antiperspirant Drug Products

(Comment 1) One comment requested that FDA reconsider its position that OTC drug monographs are substantive, as opposed to interpretive, regulations.

The agency addressed this issue and reaffirms its conclusions as stated in

paragraphs 85 through 91 of the preamble to the procedures for classification of OTC drug products (May 11, 1972, 37 FR 9464 at 9471 to 9472) and in paragraph 1 of the preamble to the TFM in the present proceeding (47 FR 36492 at 36493).

(Comment 2) Three comments disagreed with the agency's proposed definition of an antiperspirant: ''A drug product that, when applied topically to the underarm, will reduce the production of perspiration (sweat) at that site,'' (47 FR 36492 at 36503). One comment contended it was unduly restrictive and unnecessary to limit use only in the underarm area because it is not the only area of the body upon which these products could potentially be applied. The comment asked the agency to modify the definition to parallel the pharmacologic activity of the active ingredients and suggested: ''A drug product that, when applied topically, will reduce the production of perspiration (sweat) at that site.''

A second comment stated that the definition limiting use to the underarm only would adversely affect its products labeled for use on the hands and for use with orthotic and prosthetic appliances (to keep appliance-skin contact areas dry). Noting that the agency and the Panel recognized the similarities and differences between axillary and foot perspiration, a third comment stated that ingredients effective in the underarm area are probably effective to control foot perspiration.

The agency agrees with the first comment that it is not necessary to specify the area of use on the body in the definition of an antiperspirant because that information is included in the product's labeling. Accordingly, the agency is deleting the phrase ''to the underarm'' from the definition of an antiperspirant in § 350.3 (21 CFR 350.3) of this final monograph to read: ''*Antiperspirant.* A drug product applied topically that reduces the production of perspiration (sweat) at that site.'' The use of an antiperspirant on other areas of the body, as mentioned by the second and third comments, is discussed in section II.A, comment no. 4 and section II.C, comment 14 of this document.

(Comment 3) One comment stated that the TFM for OTC antiperspirant drug products was substantively and procedurally defective because it failed to address adequately the Panel's Category III recommendations concerning ''enhanced duration of effect'' and ''problem perspiration'' and failed to state what testing was required to substantiate these claims. The comment requested that FDA issue a new or amended TFM to address these issues.

The agency has determined that there is no need to withdraw, amend, or initiate a new TFM. Since the Panel's report was published in 1978, the procedural regulations for the OTC drug review were revised to comply with the Court ruling in *Cutler* v. *Kennedy*, 475 F. Supp. 838 (D.D.C. 1979). The revised regulations (46 FR 47730, September 29, 1981) provide that TFMs and final monographs will no longer contain recommended testing guidelines. The agency is not required by statute or regulation to include testing guidelines as part of OTC panel reports or TFMs. The agency stated in proposed § 350.60 of the TFM (47 FR 36492 at 36504) and states in § 350.60 of this final monograph (21 CFR 350.60) that ''To assure the effectiveness of an antiperspirant, the Food and Drug Administration is providing guidelines that manufacturers may (emphasis added) use in testing for effectiveness.''

The ''enhanced duration of effect'' and the ''problem perspiration'' issues are discussed in section II.C, comments 10 and 12 of this document. Extended duration of effect claims have been placed in Category I based on data submitted by other comments (see also comment 12). The agency has determined that claims for problem perspiration are outside the scope of this monograph because no data were submitted to support such claims (see also comment 10).

(Comment 4) One comment contended that the proposed monograph would have a disastrous economic effect on its company, which markets an antiperspirant product first formulated in 1902 and labeled for excessive perspiration, including keeping the hands free of perspiration (labeled for use on the hands for tennis, racquetball, bowling, football, and other sporting uses), and marketed for prosthesis and orthotic use (for amputees to keep their appliance contact areas dry).

To qualify for exemption from the ''new drug'' definition under the 1938 grandfather clause of the act, the drug product must have been subject to the Food and Drug Act of 1906, prior to June 25, 1938, and at such time its labeling must have contained the same representations concerning the conditions of its use (21 U.S.C. 321(p)(1)). Under the 1962 grandfather clause of the act, a drug product which on October 9, 1962 was: (1) Commercially used or sold in the United States; (2) not a ''new drug'' as defined in the 1938 act; and (3) not covered by an effective NDA under the 1938 act, would not be subject to the added requirement of effectiveness ''when intended solely for use under conditions prescribed, recommended, or suggested in labeling with respect to such drug on that day.'' (Public Law 87–781, section 107(c)(4), 76 Stat. 788, note following 21 U.S.C. 321).

The person seeking to show that a drug comes within a grandfather exemption must prove every essential fact necessary for invocation of the exemption. See *United States* v. *An Article of Drug* * * * ''*Bentex Ulcerine*,'' 469 F.2d 875, 878 (5th Cir. 1972), *cert. denied*, 412 U.S. 938 (1973). Furthermore, the grandfather clause will be strictly construed against one who invokes it. See *id.*; *United States* v. *Allan Drug Corp.*, 357 F.2d 713, 718 (10th Cir.), *cert. denied*, 385 U.S. 899 (1966). A change in composition or labeling precludes the applicability of the grandfather exemption. See *USV Pharmaceutical Corp.* v. *Weinberger*, 412 U.S. 655, 663 (1973).

Although the comment stated that its drug products have been marketed since 1902 with hand perspiration labeling claims, no evidence was submitted to show that the labeling and composition of the products have remained unchanged since either 1938 or 1962, so that they qualify as grandfathered products. The agency requested product labeling from these years on several occasions (Refs. 1, 2, and 3), but none was ever provided. Without such evidence, the products do not qualify for either grandfather exemption. The burden of proof with respect to the grandfather exemption is not on FDA, but on the person seeking the exemption. See *An Article of Drug* * * * ''*Bentex Ulcerine*,'' *supra*.

The 1938 and 1962 grandfather clauses apply only to the new drug provisions of the act (see 21 CFR 314.200(e)) and not to the adulteration and misbranding provisions. The OTC drug review was designed to implement both the misbranding and the new drug provisions of the act. (See § 330.10 (21 CFR 330.10), 37 FR 9464 at 9466.) The grandfather clauses do not preclude the agency from reviewing any currently marketed OTC drug product, regardless of whether it has grandfather protection from the new drug provisions, in order to ensure that it is not misbranded.

Although the comment claimed this final rule would have a disastrous economic effect on its company if antiperspirants can be labeled only for underarm use, it provided no documentation about this impact. The agency notes that while the company's products would need to be relabeled to bear different indications, as long as the monograph conditions are met, the

products could remain in the marketplace after relabeling occurred. The economic impact of this final rule is discussed in section VI of this document.

B. General Comments on Labeling of OTC Antiperspirant Drug Products

(Comment 5) Several comments contended that FDA should not incorporate the ''exclusivity policy'' in the final monograph by prescribing specific labeling terminology to the exclusion of other truthful nonmisleading language.

After these comments were submitted, in the **Federal Registers** of May 1, 1986 (51 FR 16258) and March 17, 1999 (64 FR 13254), the agency published final rules changing its labeling policy for stating the indications for use of OTC drug products. Under § 330.1(c)(2) (21 CFR 330.1(c)(2)), the agency provides options for labeling OTC drug products. The final monograph in this document is subject to the labeling provisions in § 330.1(c)(2). In addition, the monograph labeling follows the format and content requirements of § 201.66 (21 CFR 201.66).

(Comment 6) One comment objected to limiting the terms proposed in § 350.50(b)(1), (b)(2), and (b)(3) to ''reduces,'' ''decreases,'' ''diminishes,'' and ''lessens.'' The comment stated that ''lower'' and ''mitigate'' are synonyms for ''reduce'' and other words and phrases state, truthfully and accurately, the effect of antiperspirants.

Several comments disagreed with the agency that words such as ''stop,'' ''check,'' ''halt,'' ''end,'' ''eliminate,'' and ''protect'' should not be used in the labeling of antiperspirant drug products, even if preceded by the word ''helps,'' because these words imply the ability to stop underarm perspiration totally and would therefore mislead the consumer about the effectiveness of antiperspirant drug products. The comments mentioned the minority Panel position that ''The Panel did not see scientific data to indicate that a consumer can differentiate between such words as 'halts,' 'checks,' 'stops,' and 'ends,' as disallowable words versus 'diminishes' and 'reduces' as allowable words,'' (43 FR 46694 at 46725). One comment agreed with the minority because a review of the entire record of this proceeding found no studies or data to support a decision to disallow ''protects,'' ''halts,'' ''checks,'' and ''stops.'' Another comment requested a hearing on this issue.

One comment disagreed with the Panel's Category II status for the following labeling claims (43 FR 46694 at 46724): ''Dry,'' ''dry formula,'' ''super dry,'' ''helps stop wetness,'' ''completely guards your family,'' ''helps stop embarrassing perspiration wetness,'' ''complete protection,'' ''really helps keep you dry,'' and ''gentle enough for sensitive areas of the body.'' The comment asked the agency to allow these claims in the final monograph.

The agency has re-evaluated these claims in light of the comments' arguments and its current policy to provide consumer friendly OTC drug product labeling. The agency is deleting one previously proposed word (''diminishes'') and adding some more consumer-friendly words (''sweat'' and ''sweating'') to antiperspirant product labeling.

The agency proposed the word ''diminishes'' in § 350.50(b) as one of the optional terms that could be used as the first word of the indications statement. While the word ''diminish'' means to ''reduce,'' the agency does not consider it as consumer-friendly as the other optional words ''reduces,'' ''decreases,'' or ''lessens.'' Therefore, the agency is not including ''diminishes'' in § 350.50(b) of this final monograph as an FDA-approved term. The agency rejected the words ''mitigate'' and ''lower'' in the TFM (comment 14, 47 FR 36492 at 36496 to 36497). The agency's position has not changed. While the terms ''mitigate,'' ''lower,'' and ''diminishes'' are not in the monograph and the agency does not favor their use, manufacturers may use these terms, or other words or phrases that truthfully and accurately express a similar meaning, under the flexible labeling policy in § 330.1(c)(2).

The agency is not changing its position on the use of the word ''helps'' in conjunction with the words ''stop,'' ''halt,'' ''check,'' ''end,'' and ''eliminate.'' In the TFM (comment 14), the agency stated that these words imply the ability to stop underarm perspiration totally and would therefore mislead consumers about antiperspirant effectiveness. Although neither the Panel nor the agency had any consumer comprehension studies to support a decision to disallow this information, the comments also did not provide any data to support these terms. The agency would consider these terms if data are provided to show that consumers would not be misled about the effect of antiperspirant drug products. The agency is not including ''helps protect'' before ''underarm dampness,'' ''underarm perspiration,'' or ''underarm wetness,'' because the language is not clear and could confuse consumers.

The agency is not including any ''dry'' or similar claims (''dry,'' ''dry formula,'' ''super dry,'' ''really helps keep you dry'') in this final monograph because no criteria have been established to define ''dry.'' Thus, what may be ''dry'' for one manufacturer's product may not be ''dry'' for another manufacturer's product. The agency would consider including ''dry'' claims in the monograph if appropriate criteria for such claims are developed.

The agency is not including claims such as ''complete protection'' or ''completely guards your family'' in the monograph because there is no evidence that antiperspirant drug products provide ''complete'' protection. The agency is not including the claim ''gentle enough for sensitive areas of the body'' because the words ''sensitive areas'' may imply that the product can be used on other body areas in addition to the underarm. The agency is not including the claim ''helps stop embarrassing perspiration wetness'' because what is ''embarrassing'' or ''problem'' perspiration for one individual may not be ''embarrassing'' or a ''problem'' for others. (See section II.C, comment 10 of this document.)

The agency is not including both ''perspiration'' and ''wetness'' in the same claim because it considers the duplicative wording unnecessary. The currently allowed claims are ''* * * underarm wetness'' or ''* * * underarm perspiration.'' The agency would have no objection to ''* * * underarm perspiration wetness,'' but such would have to be done under the flexible labeling provisions of § 330.1(c)(2). The agency is adding the words ''sweat'' and ''sweating'' in § 350.50(b) as other ways to describe ''wetness'' and ''perspiration,'' because consumers regularly use these terms to describe perspiration. Based on the previous discussion, the agency concludes that a hearing is not warranted on these issues.

(Comment 7) Three comments requested that OTC antiperspirant drug products be exempted from the keep out of reach of children and accidental ingestion warnings in § 330.1(g) because these products are not toxic by oral ingestion. One comment noted only one reported ingestion in 30 years of marketing antiperspirant products. Another comment stated that aerosols, in particular, should be exempt from the ingestion warning due to the characteristics of the delivery system and the warnings already required for aerosols pressurized by gaseous propellants under § 369.21 (21 CFR 369.21).

Although the comments did not submit any data to show that antiperspirant drug products are safe if ingested, the agency believes these products should not be toxic by oral

ingestion for most individuals. However, individuals with renal dysfunction or immature renal function (i.e., infants) are at a higher risk from any exposure to aluminum. Further, ingestion of the various inactive ingredients present in these products may make young children ill or cause other undesirable consequences. Without adequate proof of safety if accidental ingestion were to occur, the agency has no basis to exempt OTC antiperspirant drug products from the accidental ingestion warning.

Although aerosol antiperspirant drug products are unlikely to be accidentally ingested by most consumers, the agency notes that the product containers are similar to those used for some food products. Spraying an aerosol into the mouth and ingesting it could be more hazardous than ingesting other dosage forms of the product because of the aerosol propellants. The warnings required under § 369.21, for those drugs in dispensers pressurized by gaseous propellants, are not related to ingestion, but state the following: ''Avoid spraying in the eyes. Do not puncture or incinerate. Do not store at temperatures above 120 °F. Keep out of reach of children.'' The agency does not consider these warnings a basis to exempt aerosol antiperspirants from the accidental ingestion warning required by § 330.1(g) for topical drug products. The last statement of the warning required by § 369.21 and the first warning required by § 330.1(g) (i.e., ''Keep out of reach of children.'') are identical as of March 17, 1999 (64 FR 13254 at 13294). Section 350.50(c)(4)(ii)) of the final monograph requires aerosol antiperspirant drug products to bear the language in § 369.21. These products do not have to repeat the first general warning required by § 330.1(g) but need to have the accidental ingestion warning required by § 330.1(g).

(Comment 8) Two comments objected to the proposed warning in § 350.50(c) for aerosol antiperspirants, which states: ''Avoid excessive inhalation.'' The comments argued that the warning duplicates and gives less information than the current warning required for aerosol drug products under § 369.21.

Section 369.21 requires the following warning statement for a drug packaged in a self-pressurized container in which the propellant consists in whole or in part of a halocarbon or hydrocarbon: ''Use only as directed. Intentional misuse by deliberately concentrating and inhaling the contents can be harmful or fatal.'' The agency does not consider this warning (which addresses deliberate misuse) as being the same as a general statement warning people to avoid excessive inhalation. There are many people who would not deliberately misuse the product who should be alerted to keep away from their face and mouth and to avoid excessive inhalation. The warning appears in the final monograph in more consumer friendly language and in the new labeling format as follows: ''When using this product [bullet] keep away from face and mouth to avoid breathing it.'' (See § 201.66(b)(4) for description of a ''bullet.'')

C. Comments on Category III Effectiveness Testing

(Comment 9) Several comments objected to user perception testing to substantiate Category III effectiveness claims. (See comment 24, 47 FR 36492 at 36499.) The comments contended that the user perception test is not reliably indicative of product effectiveness and offers at best a crude index of activity that is difficult to employ for precise qualitative and quantitative evaluations. The comments considered objective gravimetric sweat collection procedures more reliable than user perception testing to assess antiperspirant activity levels and requested that user perception testing be deleted. Three comments submitted data on user perception testing of Category III claims, including extra effective, 24-hour duration, emotional sweating, and foot perspiration (see section II.C, comments 11 through 14 of this document).

The agency has determined that user-perception test data support emotional sweating, 24-hour protection, and extra effective claims. Accordingly, the agency concludes that there are sufficient data on user perception tests (including both user and independent observer perception tests) for use of antiperspirants for the underarm. No further user perception tests are necessary if an underarm antiperspirant shows at least 20 percent sweat reduction by gravimetric tests for emotional sweating and 24-hour protection claims or 30 percent sweat reduction for extra effective claims. Adequate user perception tests have not been conducted for parts of the body other than the underarms, such as the hands or feet. The agency will still require user perception and other effectiveness data to support use of antiperspirants on the hands and feet (see section II.A, comment 4 and section II.C, comment 14 of this document).

(Comment 10) Several comments objected to the Category III status of the claims ''problem perspiration'' and ''especially troublesome perspiration.'' One comment contended these claims are not inherently misleading or untruthful and many people who do not perspire heavily may, at times, consider themselves to have ''problem'' or ''troublesome'' perspiration.

Other comments objected to the agency's definition of problem perspiration as affecting the upper 5 percent of perspirerers, contending that a more realistic approach would be to let consumers define the meaning of these words by running efficacy studies on people who identify themselves as having problem or especially troublesome perspiration. One comment objected to the economic consequences of testing the top 5 percent of the population to establish a ''problem perspiration'' claim, because this could raise the price for one efficacy evaluation from the current $5,000 to $10,000 up to $200,000. The comment requested a hearing on this issue if FDA did not revise its approach.

No data were submitted to the agency to show that any OTC antiperspirant drug product is effective in reducing ''problem'' or ''especially troublesome'' perspiration. The agency is not aware of any products that currently qualify as effective for those conditions. If products are found to be effective in the future, the agency will include a definition and labeling for ''problem'' or ''especially troublesome'' perspiration in the monograph. The agency proposed in the tentative final monograph that a 30 percent reduction in sweat production in the upper 5 percent of perspirerers is necessary for a ''problem perspiration claim'' (47 FR 36492 at 36500). As discussed in section II.C, comment 9 of this document, gravimetric testing is sufficient to prove these claims. The agency would find acceptable an antiperspirant effectiveness study on a population of individuals who perceive themselves to have ''problem perspiration,'' as one comment suggested. Based on changes in the testing to support these claims, the agency concludes that a hearing is not needed.

(Comment 11) Several comments objected to the agency's proposed Category II classification of the claims ''extra strength,'' ''extra effective,'' or any other comparative effectiveness claims (see comment 19, 47 FR 36492 at 36498). The comments argued that if manufacturers can demonstrate by appropriate testing and methods of statistical analysis that one product is more effective than another, they should be permitted to so inform consumers. The comments noted that the agency had approved an NDA for an acetaminophen ''extra strength'' product and allowed sunscreen products to label

their degree of effectiveness. One comment requested a hearing on this subject.

To prove the validity of comparative claims, two comments submitted both gravimetric and perceptual data (Refs. 4 and 5). Another comment submitted gravimetric data only (Refs. 6 and 7) and stated that one study showed that a 10 percent difference in antiperspirant effectiveness can be measured with currently marketed antiperspirant products. This comment stated that adequate data (Ref. 8) had been submitted to the Panel (43 FR 46694 at 46715) to show that as differences in antiperspirant performance levels increase, larger numbers of consumers perceive the difference. These data included a chart plotting differences in sweat reduction against the percentage of subjects who noted variations in axillary wetness. The chart shows that at 20 percent sweat reduction, approximately 45 to 50 percent of the subjects noticed a difference; at 35 percent sweat reduction, approximately 60 percent noticed a difference; and at 50 percent sweat reduction, approximately 75 percent noticed a difference. The comment contended that this study confirmed the Panel's determination that the user can perceive a shift of at least 10 percent in antiperspirant effectiveness and that a product providing a 30 percent or greater sweat reduction is perceived as more effective than a standard antiperspirant. The comments requested monograph status for ''extra strength'' and ''extra effective'' claims, as qualified by gravimetric studies.

The agency has determined that some of the studies (Ref. 4) meet the Panel's ''guidelines for user perception test to be done for claims of 'extra-effective' to be classified as Category I'' (43 FR 46694 at 46730). In these studies, two solid stick antiperspirant products (containing either 10 percent or 25 percent aluminum chlorohydrate) were compared by both a gravimetric and a user perception test. In the gravimetric test, 91 female subjects used the 10-percent product, and 88 used the 25-percent product. A 17-day conditioning period with no antiperspirant use was followed by four daily applications of one of the products to a randomly selected axilla (armpit or underarm). The opposite axilla received no treatment and served as the control. Baseline sweat production was determined the first day of the test. On days two and three, the antiperspirant was applied and 1 hour later a sweat production sample was collected. On day five, 24 hours after the fourth application, a sweat production sample was collected. Both the 10- and 25-percent products were more effective than the no treatment control for all time periods according to the statistical methods (Wilcoxon signed rank test) in the agency's guidelines for effectiveness testing of OTC antiperspirant drug products (Ref. 9). Evaluation of the Z values for the two 1-hour test days and the 24-hour test day showed that both products were statistically (Wilcoxon test) at least 20 percent better than the control axilla for all time periods ($p < 0.001$ for all three cases). Thus, both products met the requirements for standard effectiveness, i.e., a minimum of 20-percent reduction in underarm perspiration. Applying the same statistical methods to a 30-percent reduction in underarm perspiration on the last 24-hour data showed that the 25-percent product was more effective than no treatment ($p < 0.001$) and, thus, met one of the extra effective criteria.

The same study design was used in the user perception test except that the subjects applied the 10-percent product under one axilla and the 25-percent product under the other axilla. On day five, 24 hours after the fourth application, the 100 female subjects were asked ''Under which arm do you feel drier?'' All subjects had a preference: 33 favored the 10-percent product and 67 favored the 25-percent product. A statistically significant number of the subjects were able to perceive that the 25-percent product was more effective than the 10-percent product ($p = 0.0005$ one-sided). This result exceeded the Panel's requirement that 58 out of 100 subjects have a preference for the test antiperspirant (43 FR 46694 at 46731). Thus, these studies showed that the 25-percent aluminum chlorohydrate met the Panel's criteria (gravimetric measurements and user perception) for an extra effective claim.

The agency has determined that the studies indicate that gravimetric testing shows an adequate difference between a standard antiperspirant (with a 20-percent reduction in sweat) and an antiperspirant with at least a 30-percent reduction in sweat, as required by the Panel, to support an ''extra effective'' claim. The agency stated in the tentative final monograph (47 FR 36492 at 36499) that once the level of activity that is perceivable by users has been established using the Panel's recommended guidelines, it will not be necessary to perform user perception testing on individual products. Accordingly, the agency concludes that no further user perception testing is necessary for an ''extra effective'' claim, which is being included in the monograph for those antiperspirant products that reduce underarm perspiration by 30 percent or more using the guidelines for effectiveness testing of antiperspirant drug products referred to in § 350.60.

The Panel placed ''extra-strength'' claims in Category II because it concluded that ''the presence of more active ingredient in an antiperspirant product cannot be used as a basis for a claim of added effectiveness because additional amounts of antiperspirant active ingredient do not necessarily result in improved product effectiveness'' (43 FR 46694 at 46724). The Panel also stated that ''the term 'extra-strength' normally refers to increased concentration of the active ingredient which would normally mean added effectiveness.'' Several comments agreed that more active ingredient may not yield more effectiveness. Thus, a product containing 20 percent of an active ingredient (compared to 15 percent) that did not provide 30 percent or more sweat reduction could not claim ''extra strength'' or ''extra effective.''

The agency does not believe that for antiperspirants the claim ''extra strength'' is as informative to consumers as the claim ''extra effective.'' The agency considers ''extra effective'' to be the key information that consumers want to know to select an appropriate antiperspirant product. The agency is including this new labeling claim in § 350.50(b)(4) of this final monograph. Based on this discussion, the agency concludes that a hearing is not needed on this subject.

(Comment 12) Several comments objected to the Panel's Category III classification of claims for enhanced duration of effect, such as ''24-hour protection,'' ''one spray keeps you comfortably dry all day,'' ''prolonged protection,'' etc. (43 FR 46694 at 46728). One comment stated that if an antiperspirant product can be shown to provide the required 20-percent reduction in perspiration under hotroom conditions for 24, 48, etc. hours after application, then duration claims have been substantiated.

Three manufacturers submitted gravimetric studies (Refs. 4, 7, 10, and 11) that used a hotroom to induce sweating and measured sweat collected in cotton pads twice over a 24-hour period. The tested ingredients showed a 20-percent or more reduction in sweat production for both collection times, which the comments contended satisfied enhanced duration claims such as ''24 hour protection'' and ''all day protection.'' One comment added that its data (Ref. 11) support a variety of product forms (cream, roll-on, solid

stick) and, thus, the enhanced duration effect is not limited to product form.

The agency has determined that the data support a claim of enhanced duration for 24 hours according to the Panel's criteria. The protocols in seven of the studies (Refs. 7 and 10) varied only slightly from the Panel's recommended protocol. Subjects in one study abstained from antiperspirant use for 2 weeks prior to the study. Subjects in the other six studies stopped using antiperspirants 4 weeks prior to the studies. The subjects were pretreated with an antiperspirant for the 5 days prior to beginning sweat collection procedures. Sweat was collected 4 and 24 hours following the last antiperspirant application. Five studies included untreated axilla controls, and two studies included placebo controls. One product was tested in two different studies (one with a placebo and one without), and the results were virtually identical. The tests supported enhanced duration efficacy of 20 percent sweat reduction over the 24-hour period for aluminum zirconium tetrachloride (15.5 percent roll-on and 18.2 percent stick), zirconium tetrachloride (20 percent roll-on), aluminum chlorohydrate (6.8 percent aerosol), and aluminum chloride (20 percent solution).

Other data (Ref. 4) also supported enhanced duration of effectiveness for antiperspirant solid sticks containing 10 and 25 percent aluminum chlorohydrate. Subjects, who abstained from antiperspirant use for 17 days prior to the study, were pretreated with an antiperspirant for the 3 days prior to sweat collection, 1 and 24 hours after the last antiperspirant application. Standard hotroom and sweat collection procedures were used. Over the 24-hour period, both 10 percent and 25 percent aluminum chlorohydrate sticks reduced sweat production in the treated axilla by 20 percent compared to the untreated axilla. The 25-percent aluminum chlorohydrate product also showed a 30-percent reduction in sweat production.

Six other studies (Ref. 11) support enhanced duration claims. Most products showed a 20-percent reduction in sweat production compared to an untreated axilla for both the 4- and 24-hour evaluation periods, with several products showing a 30-percent sweat reduction. However, the studies did not identify the antiperspirant active ingredients.

The agency is including the following enhanced duration claims in § 350.50(b)(3) of this final monograph: ''all day protection,'' ''lasts all day,'' ''lasts 24 hours,'' or ''24 hour protection.'' In order to make such a claim, an antiperspirant product must reduce sweat production by at least 20 percent over a 24-hour period after application using the guidelines for effectiveness testing referred to in § 350.60. Antiperspirant products that meet the extra effective criteria (see section II.C, comment 11 of this document) over a 24-hour period can be labeled with both extra effective and enhanced duration claims (e.g., ''24 hour extra effective protection,'' ''all day extra effective protection,'' ''extra effective protection lasts all day,'' etc.). Claims of enhanced duration for more than 24 hours are nonmonograph because the agency has not received any data to demonstrate antiperspirant effectiveness for more than 24 hours according to the Panel's criteria.

(Comment 13) Several comments objected to the Panel's Category III classification of claims for control of emotional sweating, e.g., induced by tension or stress (43 FR 46694 at 46728). The comments contended that a product's antiperspirant activity is the same whether the sweat is due to thermal conditions or emotional factors. Some comments disagreed with the need for additional testing, especially consumer perception testing, to establish these claims. One comment requested a hearing.

One comment submitted clinical data (Refs. 7 and 12) which it contended showed: (1) There is a valid scientific protocol that combines a gravimetric sweat test with a word-quiz stress test to measure reduction in emotionally-induced sweat; (2) an antiperspirant is not washed from the axillae during controlled emotional stressing, and excessive sweat does not diminish antiperspirant effectiveness; (3) an antiperspirant effective in reducing thermally-induced sweat is effective in reducing emotionally-induced sweat also; and (4) an antiperspirant that reduces emotionally-induced sweat by 20 percent or more meets the standard for antiperspirant effectiveness for which user perception and benefit has already been accepted and, thus, there is no need for additional user perception testing. The studies included aerosol, roll-on, and stick products containing aluminum chlorohydrate or aluminum zirconium tetrachlorohydrate, the major antiperspirant active ingredients.

The agency has determined that gravimetric sweat tests combined with mental stress tests support an emotionally-induced sweating claim. The data included 12 studies with the same design of 5 days each on panels of approximately 25 female subjects: Pretest-abstention from all antiperspirants for at least 4 weeks prior to the study; day one—pretreatment control sweat collection under no stress; day two—pretreatment control sweat collection under emotional stressing; days two through five—apply test product; and days four and five—posttreatment sweat collection under emotional stressing. Subjects applied the antiperspirant test formulation to one axilla and used either a comparative formulation, a control placebo formulation, or no treatment on the opposite axilla. A control emotional challenge test, which lasted for about 60 minutes, was done on day two and an emotional challenge test was done on days four and five of the study.

Emotional sweating was induced by having subjects do a word definition test conducted by a moderator experienced at insuring optimum stress. The subjects received monetary rewards for a correct definition, but forfeited some of their rewards for incorrect or untimely definitions. Subjects had a 5-second time limit to begin a response and a 15-second maximum time to give the actual word definition. After 60 minutes, sweat was measured gravimetrically from the preweighed absorbent pads. Standard sweat collection and statistical evaluation procedures were used. The median sweat output for the 12 studies was 1,257 milligrams (mg) for the pretreatment control under emotional stressing compared to 415 mg for the pretreatment control under no stress. This word definition test effectively elicited a sweat response.

In the 12 studies using the word definition test, there was at least a 20-percent reduction of sweat production. The top 10 percent of heavy sweaters from each study (25 subjects) having the highest sweating rates on the untreated axilla had a 36.8 percent average sweat reduction compared to 38.2 percent reduction in the remaining 90 percent of each population (196 subjects), showing no significant difference in effectiveness in the two groups. Majors and Wild (Ref. 13) obtained similar results when comparing individual percent reduction in thermal sweating in the antiperspirant-treated axilla to rate of sweating from the untreated axilla in 89 subjects. They found that heavy sweating did not affect the rate of reduction.

The products tested under the emotional sweat protocol were also evaluated under a standard thermal sweat protocol at 100 °F with 30 percent relative humidity. The average percent sweat reduction for aerosols was 37.0 percent for emotional sweating and 34.0 percent for thermal sweating, for sticks it was 46.0 percent for emotional

sweating and 41.4 percent for thermal sweating, and for roll-ons it was 51.3 percent for emotional sweating and 53.3 percent for thermal sweating. These data show that the same products have similar average percent sweat reduction for both emotional and thermal sweating.

The agency concludes that gravimetric sweat tests combined with mental stress tests are sufficient to show effectiveness for control of emotionally-induced sweating; the data show antiperspirant drug products that are effective for thermal sweating are also effective for emotional sweating. The agency has determined that no additional testing (e.g., user perception tests) is required for an emotionally-induced sweating claim for products containing monograph ingredients that meet the guidelines for effectiveness testing of antiperspirant drug products referred to in § 350.60.

The agency is including the following emotionally-induced sweating claim in § 350.50(b)(2) of this final monograph: ''also [select one of the following: 'decreases,' 'lessens,' or 'reduces'] underarm [select one of the following: 'dampness,' 'perspiration,' 'sweat,' 'sweating,' or 'wetness'] due to stress''. Based on the previous discussion, the agency concludes that a hearing is not needed on this subject.

(Comment 14) One comment requested monograph status for 25 percent aluminum chlorohydrate to control foot perspiration based on gravimetric and perceptual data from four randomized, double-blind, bilateral, paired-comparison trials, each having 12 female subjects (Ref. 14). Treatment was randomly assigned; aluminum chlorohydrate was used on one foot and placebo on the other foot. A 25 percent aluminum chlorohydrate solution in 50 percent ethanol:50 percent water and a placebo control consisting of 50 percent ethanol:50 percent water were used in the first study. The same solutions in aerosol form were used in the other three studies. The procedure in the agency's ''Guidelines for Effectiveness Testing of OTC Antiperspirant Drug Products'' (Ref. 9) was modified for foot testing: (1) A 3-day pre-treatment period during which subjects were not to use any foot care products, with each subject receiving four daily product applications prior to final hotroom posttreatment testing collection; (2) sweat collection media were cotton socks rather than absorbent pads; (3) a required 5-minute period of mild exercise (walking around the hotroom at the beginning of each collection period); and (4) a modified method to calculate effectiveness due to the erratic rate of sweat collections for both treated and control feet.

The comment stated that the calculation technique included in the agency's guidelines could not be used for the following several reasons: (1) The increased number and higher concentration of sweat glands in the foot area, (2) the occlusive nature of the foot area, and (3) the erratic rate of sweat collections for both treated and control feet. The comment contended that by considering the baseline, the posttreatment sweat collections, and the preferential subject perception data, statistically significant differences could be shown between sweat collection values for the treated foot compared to baseline values.

The comment stated that based on at least a 5-percent difference between the measured sweat output of each foot, sweat reduction was achieved for the treated foot in 25 of 48 subjects (52 percent) compared to only 10 of 48 subjects (21 percent) for the control foot. The comment added that, based on the user perception questionnaire, 75 percent of the subjects (29 out of 39 subjects who were able to discriminate) were able to perceive after the hotroom exposure that the treated foot was drier compared to only 21 percent of the subjects (10 out of 48) who perceived the control foot to be drier.

A second comment submitted a proposed clinical protocol (Ref. 15), but never submitted any clinical data.

The agency has found the data are insufficient to support a foot antiperspirant claim. In axillary sweating tests submitted to the Panel, the range of effectiveness (average percent sweat reduction) of antiperspirants was 20 to 40 percent in most tests, with aerosols having a reduction range of 20 to 33 percent (43 FR 46694 at 46713). In the comment's studies on aluminum chlorohydrate for foot antiperspirancy (Ref. 14), the average percent sweat reduction was below 10 percent, which is considerably below the 20 percent minimum level of sweat reduction recommended by the Panel for efficacy testing of OTC antiperspirant drug products on the foot (43 FR 46728). In addition, the agency has a number of concerns about the comment's data treatment methods: (1) The particular sweat collections selected for analysis were not chosen consistently across studies but were based on arbitrarily chosen final sweat measurements that varied with the different studies, (2) the choice of a 5-percent difference between the measured sweat output of each foot as ''clinically significant'' seems arbitrary and was not prespecified in the protocol, (3) the efficacy criterion used (greater than 15 percent reduction from baseline) was apparently defined after the data were collected and the results are therefore potentially biased, and (4) comparison with baseline is not an adequate basis upon which to conclude product efficacy because it ignores placebo and time effects that are accounted for in between product comparisons. The agency's analysis of ''across study'' data (using the average of the two sweat collections on day four, or average of the four collections on day four and five as the baseline, and the average of the two final collections as a measure of the final sweat product) did not show a statistically significant mean (or mean percent) sweat reduction from baseline in treated or control feet.

The agency does not agree with the comment's evaluation of its user perception data, but considers the product as ineffective both in subjects who preferred placebo and in subjects with no preference. It appears that the comment chose to ignore tied preferences. However, when subjects with no preference were included in the analysis, 22 out of 48 subjects (45.8 percent) and 29 out of 48 subjects (60.4 percent) preferred the treated foot, before entering and after leaving the hotroom, respectively. Both proportions are not significantly different from 1/2 (two-tailed, $p = 0.28$ and 0.15, respectively). Furthermore, the subjects apparently could not perceive which foot, treated or untreated, was drier. More subjects failed to choose the drier foot, than chose it correctly, both at baseline and posttreatment. Thus, the wetness perception study failed to show that subjects are able to tell marginal differences in sweating of the feet.

The agency has concluded that no statistically significant treatment effect was found in sweat reduction or in subject's perception of sweat (Ref. 16). Thus, 25 percent aluminum chlorohydrate has not been shown to be an effective foot antiperspirant. The agency provided the second comment suggestions on its protocol; a revised protocol was acceptable (Ref. 17), but no test data were ever submitted. The agency is not including foot antiperspirancy claims in the final monograph.

D. Comments on Testing Guidelines

(Comment 15) Several comments requested that the background section of the effectiveness testing guidelines include the following: ''FDA recognizes that alternative methodologies may be appropriate to qualify an antiperspirant drug product as effective. These

guidelines do not preclude the use of alternative methodologies that provide scientifically valid results.''

The agency is adding this statement (but changing the words ''alternative methodologies'' to ''alternate methods'') and adding ''subject to FDA approval'' to provide for alternate methods and statistical evaluations of effectiveness test data.

(Comment 16) Several comments requested that the relative humidity of 35 to 40 percent in the effectiveness testing guidelines be lowered to 30 percent, the hotroom condition widely used by industry. One comment submitted the results of effectiveness studies (Refs. 7, 10, and 18) that used a hotroom operated at 30 + 3 percent relative humidity. The comment stated that 30 percent relative humidity accurately measures antiperspirant effectiveness without causing excessive discomfort to test subjects. Two other comments submitted effectiveness test data where the relative humidity in the hotroom was ''about 35 percent'' (Refs. 19 and 20) or ''35 percent ± 5 percent'' (Ref. 21).

Based on these data, the agency is revising the relative humidity range for hotroom conditions in the antiperspirant effectiveness testing guidelines from 35 to 40 percent to a range of 30 to 40 percent. Seven studies (Ref. 10) that showed an enhanced duration of effectiveness of 20 percent sweat reduction over a 24-hour period for several antiperspirant products (see also section II.C, comment 12 of this document) used a protocol (Ref. 18) in which the subjects were placed in a controlled environment with the temperature held at 100 ± 2 °F and the relative humidity held at 30 ± 3 percent. Because the subjects were able to generate at least 150 mg of sweat per axilla per 20 minute period, the agency considers the results of the gravimetric tests valid. In other studies (Refs. 20 and 21), sweating was induced by having the subjects sit in a hotroom maintained at a temperature of 100 ± 2 °F and at a relative humidity of about 35 percent or 35 ± 5 percent. These studies support claims of extra effectiveness and enhanced duration (24–hour claims). See section II.C, comments 11 and 12 of this document. To assure that test subjects sweat adequately during the hotroom test, the agency is adding the following baseline perspiration rate condition: ''*Baseline perspiration rate.* Test subjects must produce at least 100 milligrams of sweat from the untreated or placebo control axilla in a 20-minute collection in the controlled environment.''

(Comment 17) Two comments requested revision of the part of the antiperspirant effectiveness testing guidelines that involves application of a control formulation to the alternate axilla during testing. Noting that the guidelines state that the control formulation is to be ''devoid of any antiperspirant activity * * * determined in a test compared to no treatment,'' a comment contended that it should be appropriate to compare antiperspirant activity directly against an untreated axilla and, thereby, reduce the time, complexity, and cost of the testing, especially the cost of developing a control formulation ''devoid'' of antiperspirant activity. The comment requested that the testing guidelines be revised to provide for the application of a control formulation or no treatment to the other axilla of each test subject. The other comment submitted data from two studies (Refs. 22 and 23) where one antiperspirant formulation was tested against both a placebo control and an untreated axilla control with virtually identical results; therefore, a placebo control was unnecessary to evaluate product effectiveness.

The data (Refs. 22 and 23) involved an aerosol spray containing 6.8 percent aluminum chlorohydrate tested by two gravimetric sweat tests under hotroom conditions to substantiate the claim that the product provides ''all day wetness protection.'' Both studies had the same design: Day one—pretreatment control collection; days two, three, and four—application of antiperspirant; and days four and five—posttreatment sweat collection 4 and 24 hours after application. The data were evaluated using one of the statistical methods recommended in the antiperspirant testing guidelines. In one study (Ref. 22), the product was tested against a placebo aerosol in 44 subjects. The placebo was identical to the test formulation and supposedly devoid of antiperspirant activity; the formula difference was adjusted with aerosol propellant. The results were statistically significant and showed that the aluminum chlorohydrate aerosol effectively reduced sweat production by at least 20 percent more than the placebo aerosol at 4 and 24 hours after application. However, the placebo showed some antiperspirant activity. In the second study (Ref. 23), the same product was tested against an untreated axilla control in 49 subjects with statistically significant results. The aluminum chlorohydrate aerosol effectively reduced sweat production by at least 20 percent more on the treated axilla than the untreated control axilla at 4 and 24 hours after application.

The agency is unable to conclude from these data that an untreated comparator is equivalent to use of a placebo. The observed effect of a treatment (e.g., antiperspirant) may represent the sum of the pharmacological effects of the test drug and other effects associated with the intervention effort, which may include psychological effects and the effects of the excipients used in a product formulation. Although studies have been conducted in the past using no treatment for one axilla, the use of a placebo control for that axilla allows for assessment of the net treatment effects of the test article. Therefore, the agency is retaining the requirement for a placebo/vehicle control in the antiperspirant effectiveness testing guidelines.

The proposed guidelines stated that the control formulation is as similar as possible to the test formulation and devoid of any antiperspirant activity. As the placebo used in one study (Ref. 22) was not completely devoid of antiperspirant activity, the agency is revising the guidelines to state:

Hotroom procedure. (1) For gravimetric and user perception testing, treatments consist of the application of the test formulation to one axilla and the application of a placebo control formulation to the other axilla of each test subject. Except for the active ingredient, the placebo control formulation should be as similar as possible to the test formulation.

The agency concludes that this revised testing procedure will reduce the time, complexity, and cost of testing because it eliminates the cost of developing a control formulation ''devoid'' of antiperspirant activity.

E. Comments on Antiperspirant Active Ingredients

(Comment 18) Several comments noted a discrepancy in a heading in an active ingredient table in the Panel's report (43 FR 46694 at 46697), where ''Metal:Halide'' is used, and in proposed § 350.10 (47 FR 36492 at 36504), where ''Al:Cl'' is used. Two comments suggested that ''Al:Cl'' in the table heading and in § 350.10 should be changed to ''Metal:Cl,'' because the ratio range in the table is for the ratio of the ''Cl'' to either aluminum (''Al'') or aluminum plus zirconium (''Al+Zr'').

The agency notes that the ratio range designated as ''A1:Cl'' in the TFM should have been ''Metal:Halide,'' as it was in the Panel's report. The agency is not including the ratio range table in § 350.10 of this final monograph because this information is now included in the U.S. Pharmacopeia-

National Formulary (USP–NF) monographs for each active ingredient included in § 350.10, where applicable. The agency is changing the introductory text of § 350.10 to state: ''Where applicable, the ingredient must meet the aluminum to chloride, aluminum to zirconium, and aluminum plus zirconium to chloride atomic ratios described in the United States Pharmacopeia-National Formulary.''

(Comment 19) Two comments agreed with the agency that buffer components present in the compound, such as glycine or glycol, should be omitted when calculating the maximum allowable concentration of active ingredients in an antiperspirant product (47 FR 36492 at 36495). One comment noted a potential source of confusion because the active ingredients table in proposed § 350.10 included the buffer names along with the active ingredient names. To minimize confusion and to be consistent with the agency's policy regarding buffers, the comment requested the agency to remove the buffer names from the ''active ingredient'' column in § 350.10. The comment proposed a number of changes in the active ingredient section.

When the Panel first discussed terminology for aluminum chloride and aluminum chlorohydrate antiperspirant active ingredients, the buffer additives were not included (Ref. 24). Subsequently, the Cosmetic, Toiletry, and Fragrance Association (CTFA) Antiperspirant Task Force developed definitions for aluminum chlorohydrex complexes with propylene glycol or polyethylene glycol, and for aluminum zirconium chlorohydrex complexes with glycine (Ref. 25). The Panel adopted these definitions, including those for ingredients with buffered additives, in its report (43 FR 46694 at 46696 and 46697), and the agency proposed this nomenclature in the TFM (47 FR 36492). Since the comment was submitted, the USP–NF developed names for these antiperspirant active ingredients that include the names of the buffers, where applicable, and active ingredient names in this final monograph include the buffer, where applicable.

The agency considers calculation of the concentration of an antiperspirant ingredient present in a product based on the amount of anhydrous ingredient to be appropriate. Buffered antiperspirant ingredients contain the same active chemical moiety as the corresponding nonbuffered ingredients, and the antiperspirant activity of both ingredients is similar.

(Comment 20) One comment requested the agency allow concentrations of antiperspirant active ingredients above those proposed in the monograph as long as the amount of ingredient applied to the skin is not greater than the amount judged safe by the Panel. The comment noted that, in the TFM (comment no. 12, 47 FR 36492 at 36495 to 36496), the agency had disagreed with earlier comments on this issue and stated that ''the comments included no new data to show that a higher concentration of antiperspirant active ingredient marketed in a particular container would deliver no more than the amount of active ingredient judged safe by the Panel.''

The comment submitted new data from eight usage studies (Ref. 26) to support a higher (up to 35 percent) active ingredient concentration for powder roll-on antiperspirant drug products. Fifty male and female subjects, between the ages of 18 and 55, participated in each study. Subjects were given a preweighed product and instructed to use only that product, to keep a record of how many times they used it, and not to allow anyone else in the household to use the product. An average of 43 subjects completed the 1-week studies and returned their product to the laboratory where it was reweighed.

The amount of product applied with each use was calculated. The four powder roll-ons, which contained 33 percent aluminum zirconium tetrachlorohydrate, were found to deliver between 23 and 44 mg of antiperspirant ingredient per axilla per use. The other product forms (solid stick, cream, or liquid roll-on), containing 18 to 19 percent of either aluminum chlorohydrate or aluminum zirconium tetrachlorohydrate, were found to deliver between 54 and 98 mg of antiperspirant ingredient per axilla per use. The comment contended these data show that higher concentrations of active antiperspirant ingredients, as used in powder roll-on systems, deposit no more and, in fact, deposit less active ingredient than is deposited in a liquid roll-on, solid stick, or cream product containing proposed monograph concentrations of active ingredients. Thus, the comment argued that concentrations up to 35 percent of Category I active ingredients should be allowed in powder roll-on antiperspirants.

This issue was specifically brought before the Panel, which did not agree to change the maximum concentration (Ref. 27). The Panel noted that aluminum antiperspirants can be irritating, expressed concern that a small amount of a concentrated formulation may be more irritating than a large amount of a more dilute formulation, and concluded that antiperspirant products with a higher concentration would need an NDA with additional safety studies. The agency notes that increasing the concentration of aluminum antiperspirant ingredients increases the acidity of the material and irritation of the skin (Refs. 28, 29, and 30). The agency concludes that safety data are needed to show that powder roll-on dosage forms containing up to 35 percent aluminum chlorhydrates or aluminum zirconium chlorhydrates are not irritating.

Since the TFM was published, several citizen petitions have raised concerns about the amount of aluminum absorbed from topical antiperspirant drug products. (See section II.F, comment 23 of this document.) The agency has no data showing that products containing up to 35 percent aluminum chlorhydrates or aluminum zirconium chlorhydrates increase aluminum absorption and is not revising the monograph to provide for powder roll-on dosage forms containing up to 35 percent antiperspirant active ingredient, without additional safety data being provided.

(Comment 21) One comment requested monograph status for aluminum sesquichlorohydrate prepared by neutralizing aluminum chloride with magnesium hydroxide even though the aluminum to chloride (Al:Cl) ratio of the ingredient prepared in this manner does not fall within the range specified for aluminum sesquichlorohydrate in the TFM. The comment stated that during the course of the rulemaking all aluminum chlorhydrates placed in Category I were prepared by conventional techniques: Either by neutralization of aluminum chloride with aluminum monochlorohydrate or by a controlled reaction of aluminum metal with hydrochloric acid. Thus, the comment argued that it was both appropriate and convenient to characterize the various aluminum chlorhydrates in terms of their Al:Cl ratios.

The comment stated that its data showed that the reaction of aluminum chloride with magnesium hydroxide yields aluminum sesquichlorohydrate equivalent to that listed in the TFM and the neutralizer magnesium hydroxide does not contribute either aluminum or chloride ions to the neutralization process; thus, the Al:Cl ratio of aluminum sesquichlorohydrate prepared this way will always remain 0.33, the same as aluminum chloride alone. The comment was concerned because this Al:Cl ratio of 0.33 does not fall within the ratio range of 1.9 down

to but not including 1.25:1 proposed for aluminum sesquichlorohydrate in the tentative final monograph (47 FR 36492 at 36504). The comment contended that if the final product is regarded as a mixture of aluminum sesquichlorohydrate and magnesium chloride, and if the amount of chloride that serves as counter ions for the magnesium ions were subtracted from the total chloride, then the Al:Cl ratio of the aluminum sesquichlorohydrate component of the mixture would have the Al:Cl ratio specified in the TFM. The comment submitted data (Ref. 31) using gel permeation chromatography and elemental analysis of the eluates (the substance separated out by washing) to show that aluminum sesquichlorohydrate prepared by this neutralization method is chromatographically indistinguishable from that prepared by conventional methods. The comment suggested designating the ingredient prepared by the neutralization method as ''aluminum sesquichlorohydrate MAG.''

The agency does not find these analytical data sufficient to support the comment's claim that the ingredient prepared by this neutralization method is chemically equivalent in composition to aluminum sesquichlorohydrate. The chromatographic indistinguishability from aluminum sesquichlorohydrate prepared by conventional methods only demonstrates that the chromatographic method in this study is insufficient to support the claim. This result perhaps is to be expected because the gel permeation chromatographic method used in this study is based primarily on a size exclusion principle; however, the agency doubts that any chromatographic method will provide such support.

USP 23–NF 18 Fifth Supplement (Ref. 32) added a monograph for aluminum sesquichlorohydrate and described it as consisting of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. Its chemical formula is stated as: $Al_y(OH)_{3y-z}Cl_z.nH_2O$.

According to the method described in the comment, when aluminum sesquichlorohydrate is prepared by the reaction of aluminum chloride with magnesium hydroxide, the product must be a mixture of aluminum sesquichlorohydrate and magnesium chloride. The agency does not consider it suitable from a technical point of view to simply designate this material as aluminum sesquichlorohydrate. Information provided by the comment shows that the alternate process material is not ''equivalent in composition'' because the aluminum to chloride ratio of 0.33 is outside the specified range for aluminum sesquichlorohydrate and because the material contains measurable amounts of magnesium. Also, as discussed in section II.E, comment 18 of this document, because the atomic ratio range should be metal to halide, magnesium should be counted as a metal in the atomic ratio range of the comment's material. Using the name aluminum sesquichlorohydrate for an ingredient prepared by neutralization of aluminum chloride with magnesium hydroxide would be misleading because this would imply that the drug is the same identifiable ingredient as aluminum sesquichlorohydrate prepared by neutralization of aluminum chloride with aluminum chlorohydrate. The agency believes the material described in the comment should be classified as a new ingredient, perhaps an aluminum magnesium chlorohydrate, rather than aluminum sesquichlorohydrate.

The agency concludes that additional information on the chemical characterization of the proposed material, particularly its ionic structure, is needed to permit a more scientific review. The submitted information does not provide a technical basis for allowing the substitution of aluminum sesquichlorohydrate manufactured by neutralization with magnesium chloride for that neutralized with aluminum monochlorohydrate. The USP–NF monograph (Ref. 32) does not contain information to characterize or identify an aluminum sesquichlorohydrate containing magnesium (e.g., no identification or content test, and no assay involving magnesium calculations).

Further, the agency notes that no clinical efficacy data were provided to show that the material proposed in the comment would be equally effective as aluminum sesquichlorohydrate prepared in the conventional manner. Even minor variations in formulation, such as the addition of emollients or buffers, can alter the effectiveness of an antiperspirant ingredient. (See comment no. 8 in the TFM (47 FR 36492 at 36494).) The new mixture may be just as effective. However, whether such a finding would apply to equal amounts, or whether an equivalent effect could be achieved with a greater or lesser amount of aluminum sesquichlorohydrate prepared with magnesium hydroxide, should be determined by effectiveness testing that follows the guidelines referred to in § 350.60 of the final monograph. The agency needs appropriate effectiveness data and an appropriate USP–NF monograph amendment (see 21 CFR 330.14(i)) before the ingredient prepared by the new method can be generally recognized as safe and effective and included in the final monograph.

(Comment 22) One comment objected to the agency's rejection of its earlier request (discussed in comment no. 9 of the TFM, 47 FR 36492 at 36495) that combinations of two or more Category I antiperspirant ingredients should be Category I. The comment stated that the combination policy in § 330.10(a)(4)(iv) allows combinations of two or more safe and effective active ingredients; thus, the Panel should be reversed.

In the TFM (47 FR 36495), the agency concurred with the Panel (43 FR 46694 at 46718) that both combinations of antiperspirant active ingredients and combinations of antiperspirant active ingredients with other types of active ingredients (except for a deferred antiperspirant/antifungal combination) are Category II because of no information on the existence of any such combinations or any data to support their safe and effective use.

The agency classified antiperspirant/antifungal combination drug products in Category III in the TFM for OTC antifungal drug products (December 12, 1989, 54 FR 51136 at 51148 and 51149). No additional data were submitted to support this combination, and in the final monograph for OTC antifungal drug products (September 23, 1993, 58 FR 49890 at 49891), the agency classified all antifungal combination drug products as nonmonograph.

The comment did not provide any supporting data or specific examples of Category I antiperspirant ingredients that would be suitable for use in combination with other antiperspirant or nonantiperspirant Category I ingredients. Thus, the combination policy does not apply. These combinations remain nonmonograph. However, new clinical data may be submitted to support safety and effectiveness.

F. Comments on the Safety of Aluminum Ingredients

(Comment 23) The information and arguments presented by the citizen petitions that questioned the safety of aluminum-containing ingredients in OTC antiperspirant drug products and the comment that disagreed with one of the citizen petitions were discussed in detail in the **Federal Register** of March 23, 1993 (58 FR 15452 at 15453 and 15454). One petition was concerned that aluminum can be absorbed and get into the blood and that some of the aluminum in the blood enters the brain,

where it remains and accumulates. The petition cited a study by Perl and Good (Ref. 33) that suggested that inhaled aluminum compounds could have a direct nasal-olfactory pathway to the brain. The other petition contended that two inhalation studies (Refs. 34 and 35) provided by industry showed aluminum absorption in the peribronchial lymph nodes, brain, and adrenal glands of the animals after 12 and 24 months. Both petitions expressed concern about the potential neurotoxicity of aluminum upon chronic use, especially a possible link to Alzheimer's disease.

The comment that disagreed with one petition contended that the majority of the petitioner's references described findings from in vitro studies that did not consider the blood-brain barrier, which is the brain's main defense against potentially toxic substances such as aluminum. The comment contended that extraordinarily high concentrations of aluminum were used in these studies, and that aluminum from antiperspirants would never reach a biologically significant level to be of concern. The comment stated that the majority of researchers investigating the etiology of Alzheimer's disease would consider current evidence insufficient to link aluminum to Alzheimer's disease. The comment concluded that current scientific information does not support the need to reclassify the safety of aluminum-containing antiperspirants.

The agency does not find the current evidence sufficient to conclude that aluminum from antiperspirant use results in Alzheimer's disease. Both petitions mention the widely quoted study by Perl and Good (Ref. 33) as showing that inhaled aluminum compounds may get directly into the brain by a nasal-olfactory pathway. The agency does not consider this animal study (published as a one-page Letter to the Editor in *Lancet*) as adequate to establish a direct nasal-olfactory pathway for aluminum. This study was only a small pilot animal study, about which the agency has a number of concerns.

First, the method of introducing the aluminum to these animals was not physiologically relevant. Two strips of Gelfoam (absorbable gelatin sponge, USP) saturated with high concentrations of aluminum salts (15 percent aluminum lactate or 5 percent aluminum chloride) were inserted into rabbits' left nasal recess through a hole drilled into the frontal bone. While the authors attempted to demonstrate the accessibility of aluminum from the nasal recess to the brain, the agency questions whether the normal use of antiperspirant aerosols would ever produce a high aluminum concentration in this relatively distant anatomic site. Second, the size of this study was very small (only three rabbits in each group). The agency is concerned that any error in this complicated surgical procedure to introduce the aluminum salts or in preparing the specimens for analysis could have caused a major difference in the final results. Third, the results were not consistent. Of the three animals exposed to aluminum lactate, besides the involvement of the left olfactory bulb and the cerebral cortex, only one rabbit had a lesion in the hippocampus while the other two rabbits had granulomas found in the pyriform cortex. In the group exposed to aluminum chloride, only one rabbit had a granuloma in the olfactory bulb while the other two rabbits were free of lesions. The distribution of lesions in this study was fairly random. If a nasal-olfactory pathway exists for neuronal aluminum transport, the agency believes that the distribution of these lesions should follow a more persistent anatomical pattern. In addition, the authors were unable to explain why two of the six rabbits were free of lesions. Finally, although some of the rabbits had granulomas, these lesions did not resemble the plaques or neurofibrillary tangles found in Alzheimer's disease, and none of the rabbits had any symptomatic neurologic deficit. While this study implied that access to the brain via the nasal recess may be possible under nonphysiological conditions, a direct nasal-olfactory pathway and any relationship to Alzheimer's disease cannot be established. Several other studies, which were not done with aluminum, are of no value in establishing a direct nasal-central nervous system pathway for aluminum antiperspirants.

Aluminum lactate, one aluminum salt used in this study (Ref. 33), is not included in this final monograph. Sodium aluminum lactate has been used as a buffer for aluminum sulfate in a nonaerosol dosage form, but that product is nonmonograph.

In one of the inhalation studies (Ref. 34), the life-span of the male hamsters exposed to the aluminum chlorhydrate aerosol was shorter (583 days) than that of the controls (661 days). The female hamsters exposed to aluminum chlorhydrate had a slightly longer life-span (489 days) than the controls (481 days). Male hamsters exposed to aluminum chlorhydrate coated with a high concentration of isopropyl myristate, an emollient frequently used to increase the retention on the skin of the aluminum salts used in antiperspirant products, had a life-span (646 days) comparable to the controls (661 days). Overall, these numbers do not follow a consistent pattern and could be affected by other experimental conditions.

The same petition criticized the other inhalation study (Ref. 35), contending that the results showed that the animals had suffered significant weight loss and increased terminal brain-to-body weight ratios, results it considered consistent with clinical aluminum toxicity, and that the increase in brain weight was possibly due to cerebral edema. The petition claimed that because aluminum was found to be deposited in the animals' brains, peribronchial lymph nodes, and adrenal glands, this proved that systemic absorption of aluminum had occurred and that aluminum had been transported to the brain. Other comments disagreed with the petition's argument that the rats in this study were found to have detectable aluminum levels in their brains after 12 months, contending that this finding may only be artificial considering the analytical methods used. The comments added that if aluminum did accumulate in the rats' brains, those rats should have had symptoms of neurotoxicity, which they did not have. The comments concluded that the artificial finding should be ignored.

The agency does not concur with the petition's extrapolations. The weight loss occurred only in rats and not in guinea pigs that were similarly treated. The increase in terminal brain-to-body weight ratio occurred only in the female rats at 12 months in the low- and high-dose groups. The female rats in the middle-dose group and all the males were not affected. At 24 months, this same ratio was found to increase only in the high-dose groups of both sexes; however, the increase in the female high-dose group was not statistically significant. The agency notes that all of these findings did not follow any predictable pattern or a pattern that would be expected from a dose-related or cumulative toxin exposure.

The pattern of deposition was not consistent. In the guinea pigs, aluminum was found in the peribronchial lymph nodes, but not in the adrenal glands and brains (as occurred in the rats). The agency finds it possible that aluminum absorption and deposition may be animal dependent. If this were the case, then even if the rat data were evidence of a problem, the same situation may not apply to humans. The agency is not aware of other investigators having similar results.

The petitions and the comment had different views on a study by Rollin,

Theodorou, and Kilroe-Smith (Ref. 36) in which rabbits were exposed to aluminum oxide dust for 8 hours a day, 5 days a week, for 5 months. The authors of the study found that the brains of these rabbits had a significant increase in aluminum at the end of the study. The first petition contended that this study showed that the inhalation of aluminum antiperspirants poses a special risk because this route of delivery bypasses the blood-brain barrier. The comment calculated that this study would be equivalent to a person using spray antiperspirants for approximately 10 seconds daily for 789 years to experience the same toxicity. The second petition contended that this 10-seconds-exposure assumption was incorrect because the aluminum particles in an antiperspirant aerosol remain suspended in the air for a long period of time, and the exposure will be more than the comment calculated.

The agency finds this study has a number of limitations: (1) The extraordinary high concentrations of aluminum oxide exposure in the animals, (2) the small sample size (eight animals in each group), and (3) an overlap in the standard deviations of the results obtained decreases the power and generalizability of the study. While the study shows an accumulation of aluminum in the rabbits' body tissues under certain exposure conditions, the agency does not consider the study as providing evidence of a direct nasal-olfactory pathway or that normal use of aluminum-containing antiperspirants would provide comparable results. Further, the second petition's position includes a number of assumptions, which might not occur: (1) That the place where the product is used is a confined, poor-ventilated airspace, and (2) that the user remains in the vicinity of the dispersed aerosol for a period of time during which significant inhalation would occur.

One petition claimed that an epidemiology study by Graves et al. (Ref. 37) has shown that Alzheimer's disease was associated with the use of aluminum antiperspirants and that a high incidence of amyotrophic lateral sclerosis (ALS) and Parkinson's disease in Chamorro natives of Guam, as reported by Garruto (Ref. 38), may be related to high environmental aluminum. The agency has looked closely at the Graves et al. study (Ref. 37) because it explored the association between exposure to aluminum through the lifetime use of antiperspirants and antacids and Alzheimer's disease. This was a case-control study of 130 matched pairs, where the controls were friends or nonblood relatives of the case. Subjects (cases and controls) were matched by age, sex, and the relationship between the case/control and his or her surrogate (spouse or child).

The authors mentioned that, in general, antiperspirants contain aluminum and deodorants do not, except for some deodorants marketed for women. The authors reported that there was no association between the use of ''any'' antiperspirant/deodorant and Alzheimer's disease. However, when the data were stratified by aluminum-containing antiperspirants the overall odds ratio showed a modest increase in risk and a statistically significant trend emerged between increasing lifetime use of aluminum-containing antiperspirants and the estimated relative risk of Alzheimer's disease.

The authors commented that, to their knowledge, this was the first epidemiological study of this association between antiperspirants and Alzheimer's disease, and there were several methodologic limitations that made interpretation of their results difficult. First, there were missing data because the case surrogate and the control surrogate could only recall all variables (frequency and duration of use, and product brand name) in about one-half of the matched pairs. Second, there might have been some misclassification because the analyses were based on the most common brand provided, while some subjects may have used multiple brands. Third, the authors considered the validity of the data, resulting from difficulty in learning the subjects' exposure using telephone interview methods, to be a critical limitation. Despite these limitations, the authors considered an association between aluminum-containing antiperspirants and Alzheimer's disease as biologically plausible, but concluded that their findings are provocative and, due to methodologic problems, should be considered preliminary.

Garruto (Ref. 38) described efforts to establish models of chronic motor neuron degeneration in a long-term effort to understand the cellular and molecular mechanisms of aluminum neurotoxicity. He studied foci of dementia (ALS and Parkinson's disease) in western Pacific populations. He mentioned experimental models in rabbits and cell culture as demonstrating that chronic, rather than acute, toxicity is the cause of human neurodegenerative disorders with a long latency and slow progression. However, Garruto stated that he and his colleagues had been most deficient in the design and implementation of good epidemiological studies, particularly of Alzheimer's disease and the epidemiology of aluminum intoxication per se, and described what he felt was needed for future well-designed studies.

The petitions/comment also discussed environmental exposure to aluminum, percutaneous absorption after topical use, inhaled absorption after aerosol use, aluminum neurotoxicity (and a possible relationship to Alzheimer's disease), and possible mechanisms of action. Numerous references were provided. The agency has reviewed these references and other literature published on aluminum since the petitions were submitted. Many early references were simply hypotheses and different theories that have not been adequately substantiated in humans or any animal models. A number of studies were pilot projects in a few animals, and the agency is unable to draw any definite conclusions based on the small sample sizes.

The agency notes Priest's (Ref. 39) statement that most investigators now agree that aluminum is unlikely to be implicated in causing Alzheimer's disease, whereas Rowan (Ref. 40) contended it would be considerably more correct to state that the issue is controversial. More recently, Savory et al. (Ref. 41) stated that the question whether aluminum presents a health hazard to humans as a contributing factor to Alzheimer's disease is still subject to debate.

The agency finds the literature shows the issue of aluminum toxicity and Alzheimer's disease remains controversial and is not resolved. Scott et al. (Ref. 42) reported that aluminum has been detected in Alzheimer neurofibrillary tangles, but the significance of its presence is unknown. Kasa, Szerdahelyi, and Wisniewski (Ref. 43) reported that histochemical staining showed that aluminum was present in brain samples from Alzheimer's disease victims, but the structural localization indicated that it is not primarily involved in the etiology of the disease. Candy et al. (Ref. 44) reported that data from post mortem brain examinations of patients with chronic renal failure who did not have dialysis encephalopathy suggest that it is unlikely that aluminum plays any major role in neurofibrillary tangle formation and that its role in senile plaque formation is likely to be only part of a complex cascade of changes. Savory et al. (Ref. 41) stated that the lack of agreement on the question whether the brain content of aluminum is increased in Alzheimer's disease attests to the complexity of the issue.

Savory et al. (Ref. 41) indicated that most of the data linking aluminum

exposure to Alzheimer's disease have been derived from several epidemiological studies of aluminum in drinking water, which represents only a small percentage of the total exposure. They concluded that quantification of the risk of Alzheimer's disease from other sources of aluminum (such as food additives, cosmetics, deodorants, antiperspirants, pharmaceuticals, and respiratory dusts) is needed before the total risk from all environmental sources of aluminum can be fully evaluated.

Despite Graves et al.'s acknowledgment of the limitations of their study (Ref. 37), other authors, e.g., Anane et al. (Ref. 45), report that Graves et al. found an increased risk of Alzheimer's disease with lifetime use of aluminum-containing antiperspirants after an epidemiological study. Anane et al. applied low aqueous concentrations (0.025 to 0.1 micrograms (μg)/square centimeter) of aluminum chloride ($AlCl_3.6H_2O$) to healthy shaved Swiss mouse skin for 130 days. They reported that this led to a significant increase in urine, serum, and whole brain aluminum, especially in the hippocampus area, compared to control animals. They mentioned that this percutaneous uptake and accumulation of aluminum in the brain was greater than that caused by dietary exposure to 2.3 μg per day in feed and water.

Anane et al. conducted in vitro and in vivo mouse skin studies and showed for the first time that aluminum is absorbed through mouse skin and this contributes to a greater body burden than does oral uptake. They also mentioned that several antiperspirant preparations containing $AlCl_3.6H_2O$ are applied to sensitive regions of the skin, which may increase penetration and could be an important source of body aluminum burden. Anane et al. recommended that an epidemiological study be conducted to ascertain whether use of $AlCl_3.6H_2O$-containing antiperspirants correlates with neurodegenerative disease, because such cannot be excluded based on the results of their study.

Forbes and Agwani (Ref. 46) stated that there is uncertainty about how aluminum-containing substances enter the body, but current information suggests that the skin and/or the lung are important. They mentioned that Priest (Ref. 39) noted that at least some antiperspirant sprays contain aluminum compounds of a particle size of about 1 micrometer (micron) (μ), which is ideally sized for deposition in the deep lung, and that such deposition may also be relevant for skin.

Salib and Hillier (Ref. 47) examined clinically diagnosed Alzheimer's disease patients and controls (other dementias and nondementias) and collected information to examine the association between Alzheimer's disease and aluminum occupation. They reported that manual work, such as welding, expected to be in direct contact with aluminum dust and fumes does not appear to be significantly associated with the risk of Alzheimer's disease. The authors concluded that no significant association was shown between developing Alzheimer's disease later in life and previous occupational history for all of the occupations in the study. This included both manual workers, who would be expected to have had a higher exposure opportunity to aluminum dust and fumes, and other workers at an aluminum factory. The authors concluded that neither Alzheimer's disease nor dementia in general were shown to be associated with previous aluminum occupation.

Salib and Hillier (Ref. 47), in 1996, repeated Doll's (Ref. 48) conclusions from 1993 that it is generally accepted that the delayed effects of chronic aluminum exposure have not been adequately assessed in man. Factors that govern the bioavailability, neurotoxicity, and the effect of chronic low dose exposure to aluminum compounds remain unclear. Flaten et al. (Ref. 49) stated that the lack of a readily available radioactive isotope of aluminum has been a major obstacle toward elucidating the mechanisms of absorption, distribution, and excretion of the metal.

Both Doll (Ref. 48) and Salib and Hillier (Ref. 47) stated that the possibility of a causal link between aluminum and Alzheimer's disease must be kept open until uncertainty about neuropathological evidence is resolved and the prognosis of humans exposed to aluminum by inhalation is known. Flaten et al. (Ref. 49) stated that multidisciplinary collaborative research efforts, involving scientists from many different specialities, are needed, with emphasis placed on: (1) Increasing knowledge of the chemistry of aluminum in biologic systems and determining the cellular and molecular mechanisms of aluminum toxicity, and (2) variations in neuropathology from long-term, low-level exposure to aluminum.

In summary, the literature shows that at high doses and long-term industrial exposures, aluminum can be associated with recognizable, specific neurologic effects. However, to date, the agency considers the evidence insufficient to link aluminum to Alzheimer's disease, Parkinson's disease, or ALS. Although aluminum uptake and transport by a ''nasal-olfactory pathway'' has been suggested in a nonphysiologic study in an animal model (Ref. 36), the agency is not aware of any evidence in humans that supports an olfactory-neuronal transport of aluminum to the brain.

One petition suggested that the agency require that 90 percent of the particles of an aerosol aluminum antiperspirant be greater than 50 μ (currently the requirement is between 10 and 50 μ) to reduce exposure to the upper respiratory tract. The agency notes that both Priest (Ref. 39) and Forbes and Agwani (Ref. 46) discussed a particle size of 1 μ for deposition in the deep lung. Based on current knowledge (no proof in humans of an olfactory neuronal transport of aluminum to the brain) and the lack of information on a minimum particle size to affect the respiratory tract, the agency finds no basis to impose a greater than 50μ requirement at this time. Flaten et al. (Ref. 49) stated that the possible human toxicity of aluminum has been a matter of controversy for well over 100 years. Despite many investigators looking at this issue, the agency does not find data from topical and inhalation chronic exposure animal and human studies submitted to date sufficient to change the monograph status of aluminum containing antiperspirants. The agency will continue to monitor the scientific literature on aluminum and, if new information appears, will reassess the status of aluminum-containing antiperspirants at such time.

The agency acknowledges that small amounts of aluminum can be absorbed from the gastrointestinal tract and through the skin. Assuming a person has normal renal function, accumulation of aluminum resulting from usual exposures to antiperspirant drug products (application to the underarms once or twice daily) and subsequent absorption is considered minimal. However, people with renal dysfunction have an impairment in normal renal excretion of aluminum.

Flaten et al. (Ref. 49) noted that the first human conditions generally accepted to be causally related to aluminum exposure did not occur until the 1970's, shortly after the introduction of routine dialysis therapy in persons with chronic renal failure. Dialysis encephalopathy was perhaps the first disease recognized in this population (1972, 1976). Later, fracturing osteomalacia (1977, 1978) and a microcytic hypochromic anemia (1980) were related to aluminum exposure in dialysis patients. Flaten et al. indicated that aluminum can cause encephalopathy, bone disease, and anemia in dialysis patients resulting

from the introduction of aluminum directly into the blood stream via high-aluminum dialysate or the consumption of large oral doses of aluminum-containing phosphate binders. Reduced urine production (the major route for aluminum excretion) contributes to this problem. The authors noted that, in the early 1980's, reports began to appear describing aluminum neurotoxicity and osteotoxicity in children with renal failure who were not on dialysis treatment.

The agency is concerned that people with renal dysfunction may not be aware that the daily use of antiperspirant drug products containing aluminum may put them at a higher risk because of exposure to aluminum in the product. The agency considers it prudent to alert these people to consult a doctor before using or continuing to use these products on a regular basis and is including a warning in the final monograph: ''Ask a doctor before use if you have kidney disease.''

Flaten et al. (Ref. 49) mentioned several reports of aluminum accumulation and toxicity in individuals without chronic renal failure, especially preterm infants (primarily fed intravenously), and stated that preterm infants are at risk for aluminum loading because of their immature kidney function. Term infants with normal renal function may also be at risk because of their rapidly growing and immature brain and skeleton, and an immature blood-brain barrier. Until they are 1 to 2 years old, infants have lower glomerular filtration rates than adults, which affects their kidney function. The agency is concerned that young children and children with immature renal function are at a higher risk resulting from any exposure to aluminum. Accordingly, the agency is requiring both general warnings in § 330.1(g) on all aluminum-containing antiperspirant drug products to inform parents and others to keep these products away from children, and to seek professional assistance if accidental ingestion occurs. (See also section II.B, comment 7 of this document.)

(Comment 24) One comment submitted a research paper (Ref. 50) containing the author's theories concerning how antiperspirants and aluminum in these products may be associated with breast cancer: The secretions of the apocrine sweat glands contain androgens, which are blocked by the antiperspirant and thus caused to spread internally. These androgens may be converted in the surrounding adipose tissues to estrogens, and excess estrogens have been associated with an increase in breast cancer. Alternatively, these excess androgens may interfere with the normal functioning of the hypothalamic-pituitary axis, thereby causing an imbalance of estrogen in the body. About 50 percent of breast cancers occur in the upper outer quadrant of the breast, and axillary sweat glands are anatomically very close to this site. A protein marker called GCDFP–15 (Gross Cystic Disease Fluid Protein), which is normally found only in the sweat glands, was found in the fluids of many breast cysts. The author postulated that the blocked axillary sweat glands would cause GCDFP–15 and other markers to migrate to the breast due to its proximity and gravity, and because the fetal precursors for apocrine sweat glands and mammary glands are the same, these migrated protein markers may stimulate the breast and play a role in the carcinogenic process.

The author also postulated that aluminum may play a role in the development of breast cancer because calcification of breast tissues (commonly seen in breast cancer) may be caused by a local electrolyte imbalance induced by the absorbed aluminum. The author noted that breast cancer in Japan was more than five times lower than in the United States and postulated this has occurred because Japanese women, especially the older population, do not use antiperspirants. The author noted that the breast cancer rate is currently on the rise in Japan, especially among young premenopausal women, and postulated that this is occurring because the young Japanese generation has adopted the western habit of using antiperspirants.

The agency finds these theories lack sufficient evidence. The agency notes that the amount of androgens produced by the sweat glands is relatively insignificant compared to normal physiologic amounts produced by the adrenals and the gonads. The agency is not aware of any studies that have shown an ''internal spread'' of androgens or that establish that GCDFP–15 or other protein markers are carcinogenic in humans.

The agency considers the author's views about a local electrolyte imbalance by absorbed aluminum causing breast tissue calcification inconsistent with knowledge about the calcification process. In addition, there are many benign calcifications. Finally, many proposals (e.g., diet, lifestyle changes) have been made as to why there is an increased incidence of breast cancer among Japanese women. However, there is no evidence to associate this increase with an increased use of antiperspirants. Thus, the agency concludes that there is insufficient evidence to support these theories.

(Comment 25) The agency previously assessed the carcinogenic potential of aerosolized aluminum chlorhydrate antiperspirants in comment 22 of the TFM (47 FR 36492 at 36498 and 36499). Primary lung tumors, granulomatous lesions, and macrophagic activity were evaluated in animal studies. No increase in lung tumors was seen in the low- and mid-dose rats given doses at least 100 times greater than the expected human exposure via aerosolized antiperspirants. Normal macrophage response and pulmonary fibrosis were observed at higher doses with chronic exposure. No increase in tumors was noted in guinea pigs or hamsters at any dose levels in the studies. While the agency removed aerosol antiperspirant products containing zirconium from the market because of granuloma formation (August 16, 1977, 42 FR 41374), the agency is not aware of data that indicate aluminum antiperspirants cause foreign body granulomas or pulmonary tumors.

III. Agency Changes

1. It has been agency policy since April 3, 1989 (54 FR 13480 at 13486), that before any ingredient is included in a final OTC drug monograph, it must have a compendial (USP–NF) monograph. Compendial monographs include an ingredient's official name, chemical formula, and analytical chemical tests to confirm the quality and purity of the ingredient. These monographs establish public standards for the strength, quality, purity, and packaging of ingredients and drug products available in the United States. Eighteen of the 19 antiperspirant active ingredients that the agency proposed in § 350.10 of the antiperspirant TFM (47 FR 36492 at 36504) currently have compendial monographs. Nine of the official compendial names are the same as those proposed in § 350.10, while 10 of the names have changed slightly. (See Table 1 of this document for the previous and current ingredient names.)

TABLE 1.—ANTIPERSPIRANT ACTIVE INGREDIENTS

Name in Tentative Final Monograph	Current Name
Aluminum chloride	Same
Aluminum chlorohydrate	Same
Aluminum chlorohydrex polyethylene glycol complex	Aluminum chlorohydrex polyethylene glycol
Aluminum chlorohydrex propylene glycol complex.	Aluminum chlorohydrex propylene glycol
Aluminum dichlorohydrate	Same
Aluminum dichlorohydrex polyethylene glycol complex	Aluminum dichlorohydrex polyethylene glycol
Aluminum dichlorohydrex propylene glycol complex.	Aluminum dichlorohydrex propylene glycol
Aluminum sesquichlorohydrate	Same
Aluminum sesquichlorohydrex polyethylene glycol complex	Aluminum sesquichloro-hydrex polyethylene glycol
Aluminum sesquichlorohydrex propylene glycol complex	Aluminum sesquichloro-hydrex propylene glycol
Aluminum sulfate buffered[1]	Same
Aluminum zirconium octachlorohydrate	Same
Aluminum zirconium octachlorohydrex glycine complex	Aluminum zirconium octachlorohydrex gly
Aluminum zirconium pentachlorohydrate	Same
Aluminum zirconium pentachlorohydrex glycine complex	Aluminum zirconium pentachlorohydrex gly
Aluminum zirconium tetrachlorohydrate	Same
Aluminum zirconium tetrachlorohydrex glycine complex	Aluminum zirconium tetrachlorohydrex gly
Aluminum zirconium trichlorohydrate	Same
Aluminum zirconium trichlorohydrex glycine complex	Aluminum zirconium trichlorohydrex gly

[1] Aluminum sulfate buffered with sodium aluminum lactate.

The agency is including in § 350.10 of this final monograph those antiperspirant active ingredients that currently have a compendial monograph. Only one active ingredient, aluminum sulfate buffered, does not have a current or proposed compendial monograph. While aluminum sulfate does have a compendial monograph, the buffer component, sodium aluminum lactate, does not. This buffer ingredient must also have a compendial monograph or there must be a compendial monograph for aluminum sulfate buffered in order for aluminum sulfate buffered to be included in the antiperspirant final monograph. At the present time, this ingredient is being included in § 310.545(a)(4)(ii) as a nonmonograph ingredient because the agency is not aware of any pending compendial monograph being developed. Should a compendial monograph eventually be developed, the agency will move this ingredient from § 310.545(a)(4)(ii) to § 350.10.

2. The agency is revising the format for active ingredients in § 350.10 for consistency with recent monographs: The proposed chart format is now a paragraph format listing ingredients in alphabetical order. The amount of active ingredient is stated as ''up to _____ percent'' instead of as _____ percent or less concentration.'' The information about calculating the concentration on an anhydrous basis is moved to the preamble of § 350.10. The preamble statement about aluminum to chloride and/or aluminum to zirconium ratios is revised to state: ''Where applicable, the ingredient must meet the aluminum to chloride, aluminum to zirconium, and aluminum plus zirconium to chloride atomic ratios described in the United States Pharmacopeia-National Formulary.'' The proposed ratio range table is not included in the final monograph because this information is now included in the USP–NF monographs for each active ingredient in § 350.10, where applicable.

3. The agency is expanding the indications proposed in § 350.50(b) of the TFM to provide additional uses based on new effectiveness data. The agency is also revising the uses format to make it more concise.

Because the indications proposed in § 350.50(b)(1), (b)(2), and (b)(3) of the TFM are very similar, the agency is combining them as a single indication with choices under § 350.50(b)(1): [Select one of the following: ''decreases,'' ''lessens,'' or ''reduces''] ''underarm'' [select one of the following: ''dampness,'' ''perspiration,'' ''sweat,'' ''sweating,'' or ''wetness'']. (See section II.B, comment 6 of this document.) The agency is adding a new additional indication in § 350.50(b)(2): ''also [select one of the following: 'decreases,' 'lessens,' or 'reduces'] underarm [select one of the following: 'dampness,' 'perspiration,' 'sweat,' 'sweating,' or 'wetness'] due to stress''. (See section II.B, comment 6 and section II.C, comment 13 of this document.) The agency is adding a new additional indication in § 350.50(b)(3): Select one of the following: [''all day protection,'' ''lasts all day,'' ''lasts 24 hours,'' or ''24 hour protection'']. (See section II.C, comment 12 of this document.) The agency is adding a new additional

indication in § 350.50(b)(4) that states ''extra effective''. This claim applies to products that demonstrate 30 percent or more sweat reduction using the guidelines for effectiveness testing of antiperspirant drug products referred to in § 350.60. (See section II.C, comment 11 of this document.) The agency is adding a new additional indication in § 350.50(b)(5) for products that demonstrate extra effectiveness sustained over a 24-hour period: These products may state the claims in §§ 350.50(b)(3) and (b)(4) either individually or combined, e.g., ''24 hour extra effective protection,'' ''all day extra effective protection,'' ''extra effective protection lasts 24 hours,'' or ''extra effective protection lasts all day''. (See section II.C, comment 12 of this document.)

4. The agency is revising the ''Do not apply * * *'' warning in proposed § 350.50(c)(1) to the new labeling format. The warning now reads: ''Do not use on broken skin'' and ''Stop use if rash or irritation occurs''.

5. The agency is including a warning to alert people with renal dysfunction to consult a doctor before using antiperspirants containing aluminum. The warning appears in the new labeling format and states: ''Ask a doctor before use if you have kidney disease''. (See section II.F, comment 23 of this document.)

6. The agency has revised the August 1982 Guidelines for Effectiveness Testing. The revised guidelines (dated as of the date of publication of this document) state that ''FDA recognizes that alternate methods may be appropriate to qualify an antiperspirant drug product as effective. These guidelines do not preclude the use of alternate methods that provide scientifically valid results, subject to FDA approval.'' (See section II.D, comment 15 of this document.)

The agency has revised parts of the test procedures section of the guidelines to delete the requirement that the control formulation be devoid of ''any'' antiperspirant activity. Therefore, the control formulation no longer needs to be compared to no treatment. (See section II.D, comment 17 of this document.) The agency has changed the permitted relative humidity of the hotroom conditions from 35 to 40 percent to a range of 30 to 40 percent. (See section II.D, comment 16 of this document.) The agency has added a requirement for ''baseline perspiration rate'' to assure that test subjects sweat adequately during a hotroom test: ''Test subjects must produce at least 100 milligrams of sweat from the placebo control axilla in a 20-minute collection in the controlled environment.'' (See comment 16 also.)

Because the final monograph contains 24-hour duration effectiveness claims, the agency has revised section 4(a)(4) of the guidelines to state: ''For claims of enhanced duration of effect, the test should be conducted at least two times during the period of the claim, such as 1 hour and 24 hours after the last daily treatment for 24 hour claims.'' (See section II.C, comment 12 of this document.) Because the final monograph contains ''extra-effective'' claims shown by standard gravimetric testing to have a 30-percent or more reduction in sweat, the agency has revised the guidelines to include a section on data treatment to demonstrate, with high probability, at least 50 percent of the target population will obtain a sweat reduction of at least 30 percent. (See section II.C, comment 11 of this document.)

The revised ''Guidelines for Effectiveness Testing of OTC Antiperspirant Drug Products'' are now dated as of the date of publication of this final rule and are on file in the Dockets Management Branch (address above) and on FDA's Web site at http://www.fda.gov/cder/otc/index.htm. Persons wishing to obtain a copy of the guidelines should submit a Freedom of Information (FOI) request in writing to FDA's FOI Staff (HFI–35), 5600 Fishers Lane, Rockville, MD 20857. The agency has revised § 350.60 to include this information about the guidelines.

IV. Summary of Changes from the Proposed Rule

1. The agency is modifying the definition of an antiperspirant that was proposed in § 350.3 of the TFM to delete the phrase ''to the underarm.'' (See section II.B, comment 2 of this document.)

2. The agency is revising the format for listing active ingredients in § 350.10. (See section III.2. of this document.)

3. The agency is expanding the indications for OTC antiperspirant drug products based on new data that support these additional uses (see section III.3. of this document) and is expanding the ''Guidelines for Effectiveness Testing of OTC Antiperspirant Drug Products'' to address some of these additional uses (see section III.6. of this document).

V. The Agency's Final Conclusions

The agency is issuing a final monograph establishing conditions under which OTC antiperspirant drug products are generally recognized as safe and effective and not misbranded; 18 ingredients listed in § 350.10 are a monograph condition. In the **Federal Register** of November 7, 1990 (55 FR 46914), the agency published a final rule in part 310 establishing that certain active ingredients that had been under consideration in a number of OTC drug rulemaking proceedings were not generally recognized as safe and effective. That final rule included the antiperspirant ingredients aluminum bromohydrate, aluminum chloride (alcoholic solutions), aluminum chloride (aqueous solution) (aerosol only), aluminum sulfate, aluminum sulfate buffered (aerosol only), potassium alum, and sodium aluminum chlorohydroxy lactate in § 310.545(a)(4), and was effective on May 7, 1991. In this final rule, the agency is redesignating the text of paragraph (a)(4) as paragraph (a)(4)(i), adding new paragraph (a)(4)(i) heading, and adding new paragraph (a)(4)(ii) to contain aluminum sulfate buffered with sodium aluminum lactate. Any drug product labeled, represented, or promoted for use as an OTC antiperspirant drug that contains any of the ingredients listed in § 310.545(a)(4)(i) or (a)(4)(ii) or that is not in conformance with the monograph (21 CFR part 350) may be considered a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 321(p)) and misbranded under section 502 of the act (21 U.S.C. 352). Such a drug product can not be marketed for OTC antiperspirant use unless it is the subject of an approved application under section 505 of the act (21 U.S.C. 355) and 21 CFR part 314. An appropriate citizen petition to amend the monograph may also be submitted in accord with 21 CFR 10.30 and § 330.10(a)(12)(i). Any OTC antiperspirant drug product initially introduced or initially delivered for introduction into interstate commerce after the effective date of the final rule for § 310.545(a)(4)(i) or after the compliance dates of this final rule that is not in compliance with the regulations is subject to regulatory action.

Mandating warnings in an OTC drug monograph does not require a finding that any or all of the OTC drug products covered by the monograph actually caused an adverse event, and FDA does not so find. Nor does FDA's requirement of warnings repudiate the prior OTC drug monographs and monograph rulemakings under which the affected drug products have been lawfully marketed. Rather, as a consumer protection agency, FDA has determined that warnings are necessary to ensure that these OTC drug products continue

to be safe and effective for their labeled indications under ordinary conditions of use as those terms are defined in the act. This judgment balances the benefits of these drug products against their potential risks (see § 330.10(a)).

FDA's decision to act in this instance need not meet the standard of proof required to prevail in a private tort action (*Glastetter* v. *Novartis Pharmaceuticals, Corp.*, 252 F.3d 986, 991 (8th Cir. 2001)). To mandate warnings, or take similar regulatory action, FDA need not show, nor do we allege, actual causation. For an expanded discussion of case law supporting FDA's authority to require such warnings, see ''Labeling of Diphenhydramine-Containing Drug Products for Over-the-Counter Human Use, Final Rule'' (67 FR 72555, December 6, 2002).

VI. Analysis of Impacts

An analysis of the costs and benefits of this regulation, conducted under Executive Order 12291, was discussed in the TFM for OTC antiperspirant drug products (47 FR 36492 at 36503). The one comment received is addressed in section II.A, comment 4 of this final rule and further addressed later in this section.

FDA has examined the impacts of this final rule under Executive Order 12866, the Regulatory Flexibility Act (5 U.S.C. 601–612), and the Unfunded Mandates Reform Act of 1995 (2 U.S.C. 1501 et seq.). Executive Order 12866 directs agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). Under the Regulatory Flexibility Act, if a rule has a significant economic impact on a substantial number of small entities, an agency must analyze regulatory options that would minimize any significant impact of the rule on small entities. Section 202(a) of the Unfunded Mandates Reform Act of 1995 requires that agencies prepare a written statement of anticipated costs and benefits before proposing any rule that may result in an expenditure in any one year by State, local, and tribal governments, in the aggregate, or by the private sector, of $100 million (adjusted annually for inflation). The proposed rule that has led to the development of this final rule was published on August 20, 1982, before the Unfunded Mandates Reform Act of 1995 was enacted. This final rule will not result in an expenditure in any one year by State, local, and tribal governments, in the aggregate, or by the private sector, of $100 million.

The agency concludes that this final rule is consistent with the principles set out in Executive Order 12866 and in these two statutes. Additionally, the final rule is not a significant regulatory action as defined by the Executive order. The Unfunded Mandates Reform Act does not require FDA to prepare a statement of costs and benefits for this final rule, because the final rule will not result in any 1-year expenditure that would exceed $100 million adjusted for inflation. The current inflation adjusted statutory threshold is about $110 million.

FDA has determined that this final rule will not have a significant economic impact on a substantial number of small entities. While the exact number of affected small entities is difficult to determine at any given time, the agency received only one comment from a small entity, which is discussed later in this section. This discussion explains the agency's determination that this final rule will not have a significant economic impact on a substantial number of small entities.

The purpose of this final rule is to establish conditions under which OTC antiperspirant drug products are generally recognized as safe and effective and not misbranded. This includes establishing the allowable monograph ingredients and labeling. Eighteen of the 19 active ingredients under review are included in the final monograph. The remaining ingredient could have been included had a USP–NF monograph been developed for this ingredient. If a USP–NF monograph is developed before the effective date of this final monograph, products containing this ingredient could continue to be marketed without reformulation. Without a USP–NF monograph for the ingredient, product reformulations to include a monograph antiperspirant active ingredient or discontinuation of the products will need to occur. The agency believes that this one antiperspirant active ingredient is currently in only a few products. Based on the large number of antiperspirant drug products in the OTC marketplace and the vast array of products that one known affected company currently markets, the agency considers the required reformulation or discontinuation of a few products not to be overly burdensome or substantial. The one known affected company markets at least 30 products not affected by this final rule. Only one of its products includes the active ingredient excluded under the final rule. Any company using this active ingredient has the option to: (1) Reformulate using any of the 18 active ingredients included in this final rule, (2) reformulate without this active ingredient and market the product as a deodorant, or (3) discontinue the product.

This final rule establishes the monograph labeling for OTC antiperspirant drug products and will require relabeling of all products covered by the monograph. The agency's Drug Listing System identifies approximately 200 manufacturers and 700 marketers of 1,300 OTC antiperspirant drug products containing the 19 ingredients covered by this final rule. It is likely that there are additional products that are not currently included in the agency's system. While it is difficult to determine an exact number, the agency estimates that about 1,500 OTC antiperspirant drug products will need to be relabeled based on this final rule.

The agency has been informed that relabeling costs of the type required by a final monograph generally average about $3,000 to $5,000 per stock keeping unit (SKU) (individual products, packages, and sizes). However, some of the relabeling that occurs as a result of this specific final monograph will be due to additional indications that the agency has included in the final monograph and that manufacturers will wish to add to their labeling. Assuming that there are about 1,300 to 1,500 affected OTC SKUs in the marketplace, total one-time costs of relabeling would be $3.9 million ($3,000 per SKU x 1,300 SKUs) to $7.5 million ($5,000 per SKU x 1,500 SKUs). The agency believes that actual costs will be lower for several reasons. First, many of the label changes will be made by private label manufacturers that tend to use relatively simple and less expensive labeling. Second, the agency has finalized a revised labeling format for OTC drug products in § 201.66. The agency is allowing manufacturers to incorporate the labeling changes required by this final rule along with the new general OTC drug labeling format. Thus, the relabeling costs resulting from two different but related final rules will be individually reduced by implementing both required changes at the same time.

Some relabeling costs will be further reduced because the agency is allowing up to 18 months (24 months for products with annual sales less than $25,000) for these revisions so they may be done in the normal course of business. Thus, manufacturers who

wish to add additional indications included in this final monograph can do so at their next regular printing of product labeling. Among the steps the agency is taking to minimize the impact on small entities are: (1) To provide enough time to enable entities to use up existing labeling stock, and (2) to allow the labeling changes required by this final monograph to be done concurrently with the changes required by the new OTC drug labeling format. The agency believes that these actions provide small entities substantial flexibility and reductions in cost.

The agency considered but rejected several labeling alternatives: (1) A shorter or longer implementation period, and (2) an exemption from coverage for small entities. While the agency believes that consumers would benefit from having this new labeling in place as soon as possible, a longer time period would unnecessarily delay the benefit of new labeling and a few revised formulations. Conversely, a shorter time period was also considered but rejected because it would be inflexible and more costly for the affected companies. The agency rejected an exemption for small entities because the new labeling and revised formulations, where applicable, are also needed by consumers who purchase products marketed by those entities. However, a longer (24-month) compliance date is being provided for products with annual sales less than $25,000.

One small manufacturer has indicated that it will suffer economic consequences because it will no longer be able to make claims for use of its antiperspirant products on the hands, and for prosthesis and orthotic use. However, the manufacturer did not provide sufficient data to show that its products were safe and effective for these uses and did not provide documentation to show the economic impact of this final rule on its sales. The agency notes that the company could: (1) Relabel its products to contain only the monograph indications and then remain in the marketplace, or (2) discontinue its products. While revising the product labeling may have an economic impact on a company, it will be able to continue to market its products and can use the expanded indications provided by the final monograph to try to enhance product sales.

The final rule would not require any new reporting and recordkeeping activities, and no additional professional skills are needed. There are no other Federal rules that duplicate, overlap, or conflict with the final rule.

For the reasons in this section and under the Regulatory Flexibility Act (5 U.S.C. 605(b)), the agency certifies that this final rule will not have a significant economic impact on a substantial number of small entities. Therefore, under the Regulatory Flexibility Act, no further analysis is required.

VII. Paperwork Reduction Act of 1995

FDA concludes that the labeling requirements in this document are not subject to review by the Office of Management and Budget because they do not constitute a ''collection of information'' under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501 et seq.). Rather, the labeling statements are a ''public disclosure of information originally supplied by the Federal government to the recipient for the purpose of disclosure to the public'' (5 CFR 1320.3(c)(2)).

VIII. Environmental Impact

The agency has determined under 21 CFR 25.31(a) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

IX. Federalism

FDA has analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, the agency has concluded that the rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

X. Section 369.20 Revision

Section 369.20 (21 CFR 369.20) contains a recommended warning and caution statement for OTC antiperspirant drug products under the heading ''ANTIPERSPIRANTS:'' ''Do not apply to broken skin. If a rash develops, discontinue use.'' This statement is very similar to, but not quite as extensive as, the warnings required by the final monograph: ''Do not use on broken skin'' and ''Stop use if rash or irritation occurs''. The agency is removing the entry for ''ANTIPERSPIRANTS'' under § 369.20 because it is superseded by §§ 350.50(c)(1) and (c)(2).

XI. References

The following references are on display in the Dockets Management Branch (see section I of this document) under Docket No. 78N–0064 unless otherwise stated and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Memorandum of telephone conversation between R. Bolger, C. Holland, and K. Holland, Perspi-Cura Co., and V. Miguele, FDA, in OTC vol. 1400FR, dated November 20, 1995.

2. Memorandum of fax from V. Miguele, FDA, to R. Bolger and C. Holland, Perspi-Cura Co., in OTC Vol. 1400FR, dated February 8, 1996.

3. Memorandum of telephone message from R. Bolger, Perspi-Cura Co., to V. Miguele, FDA, in OTC Vol. 1400FR, dated March 25, 1996.

4. Studies 83–0768–70 and 83–0769–70 in Comment RPT.

5. Studies S–1367, S–1617, and ST–2280/2376) in Comment No. C00039.

6. ''Antiperspirant Efficacy Study of AP10016 (Currently Marketed Roll-on Antiperspirant With Aluminum Zirconium Tetrachlorohydrate) Against AP10021 (Currently Marketed Roll-on Antiperspirant With Aluminum Chlorohydrate),'' Exhibit 24 in Comment No. C00040.

7. Comment No. LET006.

8. Majors, P. A., and F. B. Carabello, ''Presentation to the OTC Panel for Antiperspirants of the Hill Top Research Method of Antiperspirant Evaluations and General Discussion of Results Obtained,'' in OTC Vol. 140065, August 1975.

9. FDA, ''Guidelines for Effectiveness Testing of OTC Antiperspirant Drug Products,'' in OTC Vol. 140065, August 1982.

10. Exhibits 1 through 7 in Comment No. C00040.

11. ''Claim for 'Twenty Four Hour Protection' etc., Antiperspirant Tests,'' studies S–825, S–1434, S–1473, S–1518, S–1546, and S–1604, in Comment No. C00039.

12. Exhibits 9 through 20 and 22, in Comment No. C00040.

13. Majors, P. A., and J. E. Wild, ''The Evaluation of Antiperspirant Efficacy—Influence of Certain Variables,'' *Journal of the Society of Cosmetic Chemists*, 25:139–152, 1974.

14. ''New Data on Pedal Antiperspirant Activity,'' studies in Comment C00041.

15. *Pedal Antiperspirant Efficacy Evaluation*, protocol in Comments PR1 and PR2.

16. Letter from W. E. Gilbertson, FDA, to K. R. Johannes, Scholl, Inc., coded LET11.

17. Letters from W. E. Gilbertson, FDA, to R. C. Stites, Numark Laboratories, Inc., coded LET12 and LET13.

18. ''Protocol for the Clinical Evaluation of Antiperspirant Efficacy Against Thermally Induced Sweating,'' exhibit 21, in Comment No. C00040.

19. Study 83–0769–70 in Comment No. RPT.

20. ''Claim for 'Twenty Four Hour Protection' etc., Antiperspirant Tests,''

studies No. S–825, S–1367, S–1434, S–1473, S–1518, and S–1546, in Comment No. C00039.

21. Studies ST–2280/2376 in Comment No. C00039.

22. ''Twenty-Four Hour Enhanced Duration AP Efficacy Evaluation Under Thermal Stress of: A = AP10001 (Currently Marketed Aerosol Antiperspirant With aluminum chlorohydrate) Against B = AP10008, Placebo Aerosol Antiperspirant,'' Exhibit 6, in Comment No. C00040.

23. ''AP Efficacy 24 Hour Absolute Sweat Reduction Study of: AP10001 (Marketed Aerosol Antiperspirant With Aluminum Chlorohydrate),'' Exhibit 5, in Comment No. C00040.

24. Summary Minutes of the 18th Meeting of the Advisory Review Panel on OTC Antiperspirant Drug Products, in OTC Vol. 1400FR, February 26 and 27, 1976.

25. OTC Vol. 140059.

26. Comment No. C00039.

27. Transcript of the 27th Meeting of the Advisory Review Panel on OTC Antiperspirant Drug Products, pp. 75–85, included in OTC Vol. 1400FM, January 26, 1978.

28. Lansdown, A. B. G., ''Production of Epidermal Damage in Mammalian Skins by Some Simple Aluminum Compounds,'' *British Journal of Dermatology*, 89:67–76, 1973.

29. Govett, T., and M. G. DeNavarre, ''Aluminum Chlorohydrate, New Antiperspirant Ingredient,'' *The American Perfumer and Essential Oil Review*, 49:365–368, 1947.

30. ''Zirconyl Hy+droxy Chloride Antiperspirant Combinations,'' Patent No. 2,854,382, U.S. Patent Office, included in Appendix B in OTC Vol. 140037, September 30, 1958.

31. ''Characterization of Category I Aluminum Chlorhydrates and Comparison to an Aluminum Chlorhydrate Prepared With an Alternate Neutralization Agent,'' report in Comment No. C00038.

32. Fifth Supplement, USP 23–NF 18, U.S. Pharmacopeial Convention, Inc., Rockville, MD, p. 3363, 1996.

33. Perl, D. P., and P. F. Good, ''Uptake of Aluminum into Central Nervous System Along Nasal-Olfactory Pathways,'' *Lancet*, 1:1028, May 2, 1987.

34. Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, ''Inhalation Toxicology Studies of Aerosolized Products, Final Report,'' in Comment SUP.

35. Becton, Dickinson Research Center, ''Final Report on Aluminum Chlorhydrate Study,'' in Comment SUP.

36. Rollin, H. B., P. Theodorou, and T. A. Kilroe-Smith, ''Deposition of Aluminum in Tissues of Rabbits Exposed to Inhalation of Low Concentrations of A1203 Dust,'' *British Journal of Industrial Medicine*, 48:389–391, 1991.

37. Graves, A. B. et al., ''The Association Between Aluminum-Containing Products and Alzheimer's Disease,'' *Journal of Clinical Epidemiology*, 43:35–44, 1990.

38. Garruto, R. M., ''Pacific Paradigms of Environmentally Induce Neurological Disorders: Clinical, Epidemiological and Molecular Perspectives,'' *Neurotoxicology*, 12:347–377, 1991.

39. Priest, N. D., Satellite Symposium on 'Alzheimer's Disease and Dietary Aluminum', ''The Bioavailability and Metabolism of Aluminum Compounds in Man,'' *Proceedings of the Nutrition Society*, 52:231–240, 1993.

40. Rowan, M. J., ''Recent Research on the Causes of Alzheimer's Disease,'' *Proceedings of the Nutrition Society*, 52:255–262, 1993.

41. Savory, J. et al., ''Can the Controversy of the Role of Aluminum in Alzheimer's Disease be Resolved? What are the Suggested Approaches to This Controversy and Methodological Issues to be Considered?,'' *Journal of Toxicology and Environmental Health*, 48:615–635, 1996.

42. Scott, C. W. et al., ''Aggregation of Tau Protein by Aluminum,'' *Brain Research*, 628:77–84, 1993.

43. Kasa, P., P. Szerdahelyi, and H. M. Wisniewski, ''Lack of Topographical Relationship Between Sites of Aluminum Deposition and Senile Plaques in the Alzheimer's Disease Brain,'' *Acta Neuropathologica*, 90:526–531, 1995.

44. Candy, J. M. et al., ''Aluminum Accumulation in Relation to Senile Plaque and Neurofibrillary Tangle Formation in the Brains of Patients With Renal Failure,'' *Journal of the Neurological Sciences*, 107:210–218, 1992.

45. Anane, R. et al., ''Bioaccumulation of Water Soluble Aluminum Chloride in the Hippocampus After Transdermal Uptake in Mice,'' *Archives of Toxicology*, 69:568–571, 1995.

46. Forbes, W. F., and N. Agwani, ''A Suggested Mechanism for Aluminum Biotoxicity,'' *Journal of Theoretical Biology*, 171:207–214, 1994.

47. Salib, E., and V. Hillier, ''A Case-Control Study of Alzheimer's Disease and Aluminum Occupation,'' *British Journal of Psychiatry*, 168:244–249, 1996.

48. Doll, R., ''Review: Alzheimer's Disease and Environmental Aluminum,'' *Age and Ageing*, 22:138–153, 1993.

49. Flaten, T. P. et al., ''Status and Future Concerns of Clinical and Environmental Aluminum Toxicology,'' *Journal of Toxicology and Environmental Health*, 48:527–541, 1996.

50. Comments No. C46, RPT2, and RPT3.

List of Subjects

21 CFR Part 310

Administrative practice and procedure, Drugs, Labeling, Medical devices, Reporting and recordkeeping requirements.

21 CFR Part 350

Labeling, Over-the-counter drugs.

21 CFR Part 369

Labeling, Medical devices, Over-the-counter drugs.

■ Therefore, under the Federal Food, Drug, and Cosmetic Act, and under authority delegated to the Commissioner of Food and Drugs, 21 CFR Chapter I is amended as follows:

PART 310—NEW DRUGS

■ 1. The authority citation for 21 CFR part 310 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 360b–360f, 360j, 361(a), 371, 374, 375, 379e; 42 U.S.C. 216, 241, 242(a), 262, 263b–263n.

■ 2. Section 310.545 is amended by redesignating the text of paragraph (a)(4) as paragraph (a)(4)(i), by adding new paragraph (a)(4)(i) heading and paragraphs (a)(4)(ii) and (d)(34), and by revising paragraph (d)(1) to read as follows:

§ 310.545 Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.

(a) * * *

(4) * * *

(i) *Ingredients—Approved as of May 7, 1991.* * * *

(ii) *Approved as of December 9, 2004; June 9, 2005, for products with annual sales less than $25,000.*

Aluminum sulfate buffered with sodium aluminum lactate

* * * * *

(d) * * *

(1) May 7, 1991, for products subject to paragraphs (a)(1) through (a)(2)(i), (a)(3)(i), (a)(4)(i), (a)(6)(i)(A), (a)(6)(ii)(A), (a)(7) (except as covered by paragraph (d)(3) of this section), (a)(8)(i), (a)(10)(i) through (a)(10)(iii), (a)(12)(i) through (a)(12)(iv)(A), (a)(14) through (a)(15)(i), (a)(16) through (a)(18)(i)(A), (a)(18)(ii) (except as covered by paragraph (d)(22) of this section), (a)(18)(iii), (a)(18)(iv), (a)(18)(v)(A), and (a)(18)(vi)(A) of this section.

* * * * *

(34) December 9, 2004, for products subject to paragraph (a)(4)(ii) of this section. June 9, 2005, for products with annual sales less than $25,000.

* * * * *

■ 3. Part 350 is added to read as follows:

PART 350—ANTIPERSPIRANT DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
350.1 Scope.
350.3 Definition.

Subpart B—Active Ingredients

350.10 Antiperspirant active ingredients.

Subpart C—Labeling

350.50 Labeling of antiperspirant drug products.

Subpart D—Guidelines for Effectiveness Testing

350.60 Guidelines for effectiveness testing of antiperspirant drug products.

Authority: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

PART 350—ANTIPERSPIRANT DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

§ 350.1 Scope.

(a) An over-the-counter antiperspirant drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this part and each general condition established in § 330.1 of this chapter.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 350.3 Definition.

As used in this part:

Antiperspirant. A drug product applied topically that reduces the production of perspiration (sweat) at that site.

Subpart B—Active Ingredients

§ 350.10 Antiperspirant active ingredients.

The active ingredient of the product consists of any of the following within the established concentration and dosage formulation. Where applicable, the ingredient must meet the aluminum to chloride, aluminum to zirconium, and aluminum plus zirconium to chloride atomic ratios described in the U.S. Pharmacopeia-National Formulary. The concentration of ingredients in paragraphs (b) through (j) of this section is calculated on an anhydrous basis, omitting from the calculation any buffer component present in the compound, in an aerosol or nonaerosol dosage form. The concentration of ingredients in paragraphs (k) through (r) of this section is calculated on an anhydrous basis, omitting from the calculation any buffer component present in the compound, in a nonaerosol dosage form. The labeled declaration of the percentage of the active ingredient should exclude any water, buffer components, or propellant.

(a) Aluminum chloride up to 15 percent, calculated on the hexahydrate form, in an aqueous solution nonaerosol dosage form.

(b) Aluminum chlorohydrate up to 25 percent.

(c) Aluminum chlorohydrex polyethylene glycol up to 25 percent.

(d) Aluminum chlorohydrex propylene glycol up to 25 percent.

(e) Aluminum dichlorohydrate up to 25 percent.

(f) Aluminum dichlorohydrex polyethylene glycol up to 25 percent.

(g) Aluminum dichlorohydrex propylene glycol up to 25 percent.

(h) Aluminum sesquichlorohydrate up to 25 percent.

(i) Aluminum sesquichlorohydrex polyethylene glycol up to 25 percent.

(j) Aluminum sesquichlorohydrex propylene glycol up to 25 percent.

(k) Aluminum zirconium octachlorohydrate up to 20 percent.

(l) Aluminum zirconium octachlorohydrex gly up to 20 percent.

(m) Aluminum zirconium pentachlorohydrate up to 20 percent.

(n) Aluminum zirconium pentachlorohydrex gly up to 20 percent.

(o) Aluminum zirconium tetrachlorohydrate up to 20 percent.

(p) Aluminum zirconium tetrachlorohydrex gly up to 20 percent.

(q) Aluminum zirconium trichlorohydrate up to 20 percent.

(r) Aluminum zirconium trichlorohydrex gly up to 20 percent.

Subpart C—Labeling

§ 350.50 Labeling of antiperspirant drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antiperspirant.''

(b) *Indications.* The labeling of the product states, under the heading ''Uses,'' the phrase listed in paragraph (b)(1) of this section and may contain any additional phrases listed in paragraphs (b)(2) through (b)(5) of this section, as appropriate. Other truthful and nonmisleading statements, describing only the uses that have been established and listed in paragraphs (b)(1) through (b)(5) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) For any product, the labeling states [select one of the following: ''decreases,'' ''lessens,'' or ''reduces''] ''underarm'' [select one of the following: ''dampness,'' ''perspiration,'' ''sweat,'' ''sweating,'' or ''wetness''].

(2) The labeling may state ''also [select one of the following: 'decreases,' 'lessens,' or 'reduces'] underarm [select one of the following: 'dampness,' 'perspiration,' 'sweat,' 'sweating,' or 'wetness'] due to stress''.

(3) For products that demonstrate standard effectiveness (20 percent sweat reduction) over a 24-hour period, the labeling may state [select one of the following: ''all day protection,'' ''lasts all day,'' ''lasts 24 hours,'' or ''24 hour protection''].

(4) For products that demonstrate extra effectiveness (30 percent sweat reduction), the labeling may state ''extra effective''.

(5) Products that demonstrate extra effectiveness (30 percent sweat reduction) sustained over a 24-hour period may state the claims in paragraphs (b)(3) and (b)(4) of this section either individually or combined, e.g., ''24 hour extra effective protection'', ''all day extra effective protection,'' ''extra effective protection lasts 24 hours,'' or ''extra effective protection lasts all day''.

(c) *Warnings.* The labeling of the product contains the following statements under the heading ''Warnings'':

(1) ''Do not use on broken skin''.

(2) ''Stop use if rash or irritation occurs''.

(3) ''Ask a doctor before use if you have kidney disease''.

(4) *For products in an aerosolized dosage form.* (i) ''When using this product [bullet][1] keep away from face and mouth to avoid breathing it''.

(ii) The warning required by § 369.21 of this chapter for drugs in dispensers pressurized by gaseous propellants.

(d) *Directions.* The labeling of the product contains the following statement under the heading ''Directions'': ''apply to underarms only''.

Subpart D—Guidelines for Effectiveness Testing

§ 350.60 Guidelines for effectiveness testing of antiperspirant drug products.

An antiperspirant in finished dosage form may vary in degree of effectiveness because of minor variations in formulation. To assure the effectiveness of an antiperspirant, the Food and Drug Administration is providing guidelines that manufacturers may use in testing for effectiveness. These guidelines are on file in the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. These guidelines are available on the FDA's Web site at http://www.fda.gov/cder/

[1] See § 201.66(b)(4) of this chapter for definition of bullet.

otc/index.htm or on request for a nominal charge by submitting a Freedom of Information (FOI) request in writing to FDA's FOI Staff (HFI–35), 5600 Fishers Lane, rm. 12A–16, Rockville, MD 20857.

PART 369—INTERPRETATIVE STATEMENTS RE WARNINGS ON DRUGS AND DEVICES FOR OVER-THE-COUNTER SALE

■ 4. The authority citation for 21 CFR part 369 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 371.

§ 369.20 [Amended]

■ 5. Section 369.20 *Drugs; recommended warning and caution statements* is amended by removing the entry for ''ANTIPERSPIRANTS.''

Dated: May 16, 2003.

Jeffrey Shuren,

Assistant Commissioner for Policy.

[FR Doc. 03–14140 Filed 6–6–03; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 510

New Animal Drugs; Change of Sponsor's Name; Technical Amendment

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule, technical amendment.

SUMMARY: The Food and Drug Administration (FDA) is amending the animal drug regulations to reflect a change of sponsor's name from Fort Dodge Animal Health, Division of American Cyanamid Co., to Fort Dodge Animal Health, Division of Wyeth Holdings Corp. The regulations are also being revised to correct the address for Fort Dodge Animal Health, Division of Wyeth.

DATES: This rule is effective June 9, 2003.

FOR FURTHER INFORMATION CONTACT: David R. Newkirk, Center for Veterinary Medicine (HFV–100), Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855; 301–827–6967; e-mail: *dnewkirk@cvm.fda.gov*.

SUPPLEMENTARY INFORMATION: Fort Dodge Animal Health, Division of American Cyanamid Co., P.O. Box 1339, Fort Dodge, IA 50501, has informed FDA of a change of name to Fort Dodge Animal Health, Division of Wyeth Holdings Corp. Accordingly, the agency is amending the regulations in 21 CFR 510.600(c) to reflect the change.

In addition, when the name of Fort Dodge Animal Health, Division of American Home Products Corp. was changed to Fort Dodge Animal Health, Division of Wyeth (67 FR 67520, November 6, 2002), an inaccurate correction to the address was made. At this time, it is being changed to the original and correct address.

This rule does not meet the definition of ''rule'' in 5 U.S.C. 804(3)(A) because it is a rule of ''particular applicability.'' Therefore, it is not subject to the congressional review requirements in 5 U.S.C. 801–808.

List of Subjects in 21 CFR Part 510

Administrative practice and procedure, Animal drugs, Labeling, Reporting and recordkeeping requirements.

■ Therefore, under the Federal Food, Drug and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs and redelegated to the Center for Veterinary Medicine, 21 CFR part 510 is amended as follows:

PART 510—NEW ANIMAL DRUGS

■ 1. The authority citation for 21 CFR part 510 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 360b, 371, 379e.

§ 510.600 [Amended]

■ 2. Section 510.600 *Names, addresses, and drug labeler codes of sponsors of approved applications* is amended.

a. In the table in paragraph (c)(1), in the entry for ''Fort Dodge Animal Health, Division of Wyeth'' and in the table in paragraph (c)(2), in the entry for ''000856'' by removing ''500'' and by adding in its place ''800''.

b. In the table in paragraph (c)(1), in the entry for ''Fort Dodge Animal Health, Division of American Cyanamid Co.'' and in the table in paragraph (c)(2), in the entry for ''053501'' by removing ''American Cyanamid Co.'' and by adding in its place ''Wyeth Holdings Corp.''.

Dated: May 19, 2003.

Steven D. Vaughn,

Director, Office of New Animal Drug Evaluation, Center for Veterinary Medicine.

[FR Doc. 03–14303 Filed 6–6–03; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF THE TREASURY

Internal Revenue Service

26 CFR Parts 1 and 602

[TD 9059]

RIN 1545–AX18

Coordination of Sections 755 and 1060; Allocation of Basis Adjustments Among Partnership Assets and Application of the Residual Method to Certain Partnership Transactions

AGENCY: Internal Revenue Service (IRS), Treasury.

ACTION: Final regulations and removal of temporary regulations.

SUMMARY: This document finalizes regulations relating to the allocation of basis adjustments among partnership assets under section 755. The regulations are necessary to implement section 1060, which applies the residual method to certain partnership transactions.

DATES: These regulations are effective June 9, 2003.

FOR FURTHER INFORMATION CONTACT: Craig Gerson, (202) 622–3050 (not a toll-free number).

SUPPLEMENTARY INFORMATION:

Background

This document contains amendments to 26 CFR part 1 under section 755 of the Internal Revenue Code (Code). On April 5, 2000, a notice of proposed rulemaking (REG–107872–99, 2000–1 C.B. 911) under section 755 was published in the **Federal Register** (65 FR 17829). Only one commentator submitted written comments in response to the notice of proposed rulemaking, and no public hearing was requested or held. After consideration of the comment, the proposed regulations are adopted as revised by this Treasury decision.

Explanation of Revisions and Summary of Contents

1. Summary

Section 743(b) provides for an optional adjustment to the basis of partnership property following certain transfers of partnership interests. The amount of the basis adjustment is the difference between the transferee's basis in the partnership interest and the transferee's share of the partnership's basis in the partnership's assets. Once the amount of the basis adjustment is determined, it is allocated among the partnership's individual assets pursuant to section 755.

EXHIBIT 358

Review > Vaccine. 2002 May 31;20 Suppl 3:S18-23. doi: 10.1016/s0264-410x(02)00166-4.

Aluminum Salts in vaccines--US Perspective

Norman W Baylor [1], William Egan, Paul Richman

Affiliations
PMID: 12184360 DOI: 10.1016/s0264-410x(02)00166-4

Erratum in

Vaccine. 2002 Sep 10;20(27-28):3428

Abstract

Aluminum in the form of aluminum hydroxide, aluminum phosphate or alum has been commonly used as an adjuvant in many vaccines licensed by the US Food and Drug Administration. Chapter 21 of the US Code of Federal Regulations [610.15(a)] limits the amount of aluminum in biological products, including vaccines, to 0.85 mg/dose. The amount of aluminum in vaccines currently licensed in the US ranges from 0.85-0.125 mg/dose. Clinical studies have demonstrated that aluminum enhances the antigenicity of some vaccines such as diphtheria and tetanus toxoids. Moreover, aluminum-adsorbed diphtheria and tetanus toxoids are distinctly more effective than plain fluid toxoids for primary immunization of children. There is little difference between plain and adsorbed toxoids for booster immunization. Aluminum adjuvants have a demonstrated safety profile of over six decades; however, these adjuvants have been associated with severe local reactions such as erythema, subcutaneous nodules and contact hypersensitivity.

LinkOut - more resources

EXHIBIT 359

1. Thayer, D. W., G. Boyd, W.S. Muller, et al. "Radiation resistance of *Salmonella,*" *Journal of Industrial Microbiology,* 5: 383–390, 1990.
2. Memorandum for FAP 8M4584 from V. K. Bunning, FDA, to W. Trotter, FDA, April 4, 2000.
3. Memorandum for FAP 8M4584 from K. Morehouse, FDA, to W. Trotter, FDA, April 11, 2000.
4. Memorandum for FAP 8M4584 from K. Morehouse, FDA, to W. Trotter, FDA, May 14, 1999.
5. Memorandum for FAP 8M4584 from I. Chen, FDA, to W. Trotter, FDA, December 11, 1998.
6. Memorandum for FAP 8M4584 from I. Chen, FDA, to W. Trotter, FDA, March 31, 2000.
7. U.S. Department of Agriculture, Agricultural Research Service, USDA National Nutrient Database for Standard Reference, Release 23, Nutrient Data Laboratory Home Page (*http://www.ars.usda.gov/ba/bhnrc/ndl*), 2010.
8. Bureau of Foods Irradiated Foods Committee, *Recommendations for Evaluating the Safety of Irradiated Food,* Prepared for the Director, Bureau of Foods, FDA, July 1980.

Dated: April 8, 2011.

Leslie Kux,

Acting Assistant Commissioner for Policy.

[FR Doc. 2011–8815 Filed 4–12–11; 8:45 am]

BILLING CODE 4160–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 610

[Docket No. FDA–2010–N–0099]

Revision of the Requirements for Constituent Materials

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the biologics regulations to permit the Director of the Center for Biologics Evaluation and Research (CBER) or the Director of the Center for Drug Evaluation and Research (CDER), as appropriate, to approve exceptions or alternatives to the regulation for constituent materials. A request for an exception or alternative will be considered for approval when the data submitted in support of such a request establish the safety, purity, and potency of the biological product for the conditions of use, including indication and patient population, for which the applicant is seeking approval. FDA is taking this action due to advances in developing and manufacturing safe, pure, and potent biological products licensed under the Public Health Service Act (the PHS Act) that, in some instances, render the existing constituent materials regulation too prescriptive and unnecessarily restrictive. This rule provides manufacturers of biological products with flexibility, as appropriate, to employ advances in science and technology as they become available, without diminishing public health protections.

DATES: This rule is effective May 13, 2011.

FOR FURTHER INFORMATION CONTACT: Paul E. Levine, Jr., Center for Biologics Evaluation and Research (HFM–17), Food and Drug Administration, 1401 Rockville Pike, Suite 200N, Rockville, MD 20852–1448, 301–827–6210.

SUPPLEMENTARY INFORMATION:

I. Background

In the **Federal Register** of March 30, 2010 (75 FR 15639), FDA published a proposed rule to amend the regulations for constituent materials under § 610.15 (21 CFR 610.15). Constituent materials include ingredients, preservatives, diluents, adjuvants, extraneous protein and antibiotics that are contained in a biological product. FDA is amending the regulation for constituent materials to allow the Director of CBER or the Director of CDER, as appropriate, to approve an exception or alternative to the requirements under § 610.15. An exception or alternative will be considered for approval when the data submitted in support of such a request establish the safety, purity, and potency of the biological product for the conditions for which the applicant is seeking approval. Under the final rule, the Director of CBER or CDER would not approve an exception or alternative when the data or the conditions of use, including indication and patient population, for which the applicant is seeking approval, do not provide a sufficient scientific and regulatory basis for such an approval.

The final rule provides manufacturers of biological products with flexibility, as appropriate, to employ advances in science and technology, as they become available. However, the final rule does not diminish public health protections that are provided by existing laws and regulations. The final rule gives manufacturers the potential to employ advances in science and technology if the data provide a sufficient regulatory basis for approval of the product. This means that each manufacturer's request for an exception or alternative will be considered on a case-by-case basis to determine whether the product at issue meets the statutory and regulatory criteria for safety, purity, and potency for use in the intended population. The Director of CBER or CDER will only approve a request for an exception or alternative after determining that the particular request meets this prescribed criteria for the intended population. Examples of how the final rule provides flexibility (such as alternatives to the use of preservatives and modifications to the amount of aluminum permitted in certain biological products), without diminishing public health protections, are provided in the paragraphs that follow.[1]

Standards for certain constituent materials present in biological products are provided under § 610.15. Section 610.15(a) requires that all ingredients used in a licensed product, and any diluent provided as an aid in the administration of the product, meet generally accepted standards of purity and quality. Any preservative used must be sufficiently nontoxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in the combination used, it must not denature the specific substances in the product to result in a decrease below the minimum acceptable potency within the dating period when stored at the recommended temperature. Products in multiple-dose containers must contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine; Poliovirus Vaccine Live Oral; viral vaccines labeled for use with the jet injector; dried vaccines when the accompanying diluent contains a preservative; or to an allergenic product in 50 percent or more volume in volume (v/v) glycerin. Furthermore, under § 610.15, an adjuvant must not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product.

Section 610.15(a) also requires that the amount of aluminum in the recommended individual dose of a biological product not exceed:

1. 0.85 milligrams if determined by assay;

[1] Although specific examples for use of extraneous protein and antibiotics are not provided, the final rule also allows for flexibility in applying the existing standards for extraneous proteins and antibiotics (§ 610.15(b) and (c)); provided that each request for an alternative or exception to these requirements is supported by data that establish the safety, purity, and potency of the biological product.

2. 1.14 milligrams if determined by calculation on the basis of the amount of aluminum compound added; or

3. 1.25 milligrams determined by assay provided that data demonstrating that the amount of aluminum used is safe and necessary to produce the intended effect are submitted to and approved by the Director of CBER or the Director of CDER.

Section 610.15 establishes standards for the presence of certain constituent materials in licensed, biological products and/or strictly limits the amount of certain constituent materials present in licensed biological products. However, in order to employ advancements in science and technology to benefit the public health, flexibility in applying these regulatory standards is needed.

For example, § 610.15 contains specific requirements as to preservatives. Preservatives are compounds that kill or prevent the growth of micro-organisms, particularly bacteria and fungi. The current requirements for preservatives were based, at least in part, on reports from scientific literature concerning serious injuries and deaths associated with bacterial contamination of multiple-dose containers of vaccines that did not contain a preservative.[2] As discussed previously, § 610.15 provides for limited exceptions from the preservative requirement. These exceptions include live viral vaccines that had been licensed under section 351 of the PHS Act (42 U.S.C. 262) and that were in production when the National Institutes of Health (NIH) issued the 1968 regulation.[3] [4]

Preservatives in multiple-dose containers have a long record of safe and effective use in preventing microbial growth in the event that the vaccine is accidentally contaminated, as might occur with repeated punctures of a multiple-dose container. Even though the use of preservatives has significantly declined in recent years with the use of products filled in single-dose containers that do not require addition of a preservative, some biological products such as inactivated influenza virus vaccines are still presented in multi-dose containers with a preservative. The use of preservatives could also decline further as manufacturers develop and employ new technologies, such as multi-dose adaptors to prevent contamination of products in multiple-dose containers, without the use of preservative.

However, the current regulation under § 610.15(a) does not provide FDA with flexibility to consider situations (outside of the listed exceptions) in which to allow the use of preservative-free vaccines in multiple-dose containers. It is necessary for FDA to have flexibility in applying the regulatory requirements for preservatives when, for example, state-of-the art technologies, such as the development of devices to ensure aseptic withdrawing offer a safe alternative to the use of preservatives in multiple-dose containers. The final rule permits the Director of CBER or the Director of CDER to approve a request to market a biological product in multiple-dose containers without the use of a preservative, if the manufacturer demonstrates that sufficient measures, such as an aseptic withdrawing technique through the use of an appropriate device, ensure that the product continues to meet the statutory and regulatory requirements for safety, purity, and potency. Thus, the final rule allows flexibility in the use of advancements in technology to provide a public benefit, while continuing to ensure the safety, purity, and potency of the product.

Another example where it is necessary for FDA to have flexibility in applying current regulatory requirements pertains to the amount of aluminum permitted under § 610.15(a) in the recommended single human dose of a biological product. Aluminum, in the form of an aluminum salt, is used as an adjuvant in certain biological products. The existing regulation limits the amount of aluminum per dose to no more than 0.85 milligrams (mg) if determined by assay or 1.14 mg if determined by calculation on the basis of the amount of aluminum compound added. In 1981, FDA amended § 610.15(a) to increase the permissible level of aluminum per dose to 1.25 mg both to make the regulation consistent with World Health Organization standards,[5] and because it appeared that certain groups (such as renal dialysis patients), who were understood to be at high risk of contracting hepatitis, might require a higher dosage of the hepatitis B vaccine, which would in turn, require amounts of aluminum as high as 1.25 mg per dose. (See "General Biological Products Standards; Aluminum in Biological Products," 46 FR 51903, October 23, 1981. See also "General Biological Products Standards for Aluminum in Biological Products," 46 FR 23765, April 28, 1981).

The aluminum content per dose in the formulation of a licensed biological product, as specified in § 610.15(a), reflects the NIH Minimum Requirements for Diphtheria Toxoid (1947) [6] and Tetanus Toxoid (1952).[7] The final rule does not alter the existing requirements regarding the amount of aluminum in a biological product. Instead, in a change that is analogous to the one FDA issued in 1981, involving the groups who were at high risk of contracting hepatitis, the final rule allows either the Director of CBER or the Director of CDER to approve an exception or alternative when the Director determines that a biological product meets the requirements for safety, purity, and potency for the conditions for which the applicant is seeking approval, but contains an amount of aluminum that is higher than currently permitted by § 610.15. For example, the final rule permits the Director of CBER or CDER to approve a manufacturer's request for an exception to use a proposed therapeutic vaccine for treating individuals with cancer, when the proposed vaccine contains aluminum levels higher than currently allowed but still meets the requirements of safety, purity, and potency.

II. Clarifications to the Preamble of the Proposed Rule

FDA received comments on the rule from manufacturers, private and public interest groups, and the general public. In response to comments expressing concerns about the safety of a licensed product for which FDA grants an exception or alternative to current regulations, FDA emphasizes that a manufacturer's request for an exception or alternative will not be approved unless the submitted data meet the

[2] See "The National Vaccine Advisory Committee Sponsored Workshop on Thimerosal Vaccines," pp. 21–25, August 11, 1999. See also Wilson, Graham S., *Hazards of Immunization,* 1967.

[3] With the creation of NIH, NIH had regulatory authority over biological products until 1972, at which time they were transferred to FDA. NIH issued the precursor regulation to constituent materials, § 610.15, in the **Federal Register** of January 10, 1968 (33 FR 367 at 369). See the **Federal Register** notice of June 29, 1972 (37 FR 12865) and the **Federal Register** notice of August 9, 1972 (37 FR 15993), for more information concerning the transfer of authority from NIH to FDA and how the regulations pertaining to biological products under 21 CFR part 73 were transferred to the then newly established 21 CFR part 273.

[4] Biological products had contained preservatives prior to 1968. "The National Vaccine Advisory Committee Sponsored Workshop on Thimerosal Vaccines," p. 24, August 11, 1999.

[5] More specifically, the amendment permitted the use of up to 1.25 mg per dose of aluminum determined by assay provided that data demonstrating that the amount of aluminum used is safe and necessary to produce the intended effect are submitted to and approved by the Director, Bureau of Biologics. "General Biological Products Standards; Aluminum in Biological Products," (46 FR 51903, October 23, 1981).

[6] NIH, Minimum Requirements for Diphtheria Toxoid, 4th Revision, 1947.

[7] NIH, Minimum Requirements for Tetanus Toxoid, 4th Revision, 1952.

statutory and regulatory criteria for safety, purity, and potency for use in the intended population. FDA also emphasizes that the product at issue must be shown to be safe, pure, and potent for the conditions of use, including proposed indication and patient population, for which the applicant is seeking approval, in determining whether the product may be approved. FDA further clarifies that consideration for approval of a request will be done case-by-case and will be based on review of the data submitted in support of a request.

In addition, in response to comments, FDA clarifies that there is both a need for FDA to have flexibility in applying the regulatory standards in § 610.15, and a need for manufacturers to have flexibility in employing advancements in science and technology for developing new safe, pure, and potent alternatives to current products. FDA provides more discussion on the need for flexibility in the responses to comments on the proposed rule.

FDA considered all comments received in response to the proposed rule and has determined that the proposed rule should be issued as a final rule. Accordingly, FDA is issuing as a final rule the amendment to § 610.15 under paragraph (d) to permit the Director of CBER or the Director of CDER, as appropriate, to approve an exception or alternative to the regulatory requirements for constituent materials, when the data submitted with the request for approval of an exception or alternative establish the safety, purity, and potency of the biological product, and is acceptable for use in the intended population. All requirements under § 610.15 remain in effect, except those for which the Director approves an exception or alternative. FDA approval of an exception or alternative will be done case-by-case, based on the data submitted for a specific product. Manufacturers seeking approval of an exception or alternative must submit a request in writing. The request may be submitted as part of the original biologics license application (BLA) or as an amendment to the original, pending application or as a prior approval supplement to an approved application.

III. Comments on the Proposed Rule

FDA received 15 letters of comment on the proposed rule, not including 1 duplicate letter from the same commenter. As stated previously, these comments were received from manufacturers, private and public interest groups, and the general public. Several of the comments supported the proposed rule and several comments disagreed with the proposed rule. Some of the comments on the proposed rule were similar to or duplicates of other comments received, and have been grouped together, where appropriate, to facilitate a uniform response.

To make it easier to identify the comments and our corresponding responses, the word "Comment" followed by a number is placed in parentheses and is used to indicate a particular comment or set of similar comments, as appropriate. The word "Response" in parentheses precedes FDA's response to a comment. The order of comments and responses, as listed, do not represent a value assigned to the comment but is used for organizational purposes only.

(Comment 1) Several comments supported the proposed rule. One such comment praised the rule for broadening the potential capacity for biologics manufacturers to provide medicines to the public without compromising the high level expectation of demonstrating safety, purity, and potency. Another comment supported the proposed rule for providing a means to advance "innovative science" and applications of use. Yet another comment expressed interest in seeing the "reasonable flexibility" provided in the proposed rule extended to other biopharmaceutical fields. Still another comment found the conditions and recommendations in the proposed rule to be comprehensible and useful.

(Response) FDA acknowledges and appreciates the supportive comments. As previously stated, the rule allows FDA the flexibility to approve an exception or alternative to the constituent materials regulation, without diminishing public health protections. As such, the final rule provides patients safe access to important products resulting from advances in science and technology. FDA continues to review existing regulations and may propose modification of these regulations as appropriate for public health and safety.

(Comment 2) One comment requests clarification as to whether a request for an exception or alternative to the requirements under § 610.15 can be made earlier in clinical development rather than waiting until submitting the original BLA.

(Response) FDA clarifies that although a manufacturer may submit a request for an exception or alternative early in the clinical development of a biological product, FDA considers such a request to be timely when the data intended to support the request establish the safety, purity, and potency of the biological product for its intended use. In developing data necessary to support a request for an exception or alternative, manufacturers must comply with all applicable laws and regulations, including the procedures and requirements for investigational new drug applications (INDs) and BLAs under parts 312 and 601 (21 CFR parts 312 and 601). Only after FDA determines that the biological product meets the statutory and regulatory criteria for safety, purity, and potency, and is acceptable for use in the intended population, may the Director of CBER or CDER approve a request for an exception or alternative.

However, FDA strongly encourages early communication from manufacturers intending to submit a request for an exception or alternative to the requirements under § 610.15. This includes pre-IND and IND communications by which manufacturers may seek FDA advice concerning issues such as data needed to support the rationale for testing a biological product in humans, the design of nonclinical pharmacology, toxicology, and drug activity studies, initial development plans for the biological product, and regulatory requirements for demonstrating safety, purity, and potency. Early communications between FDA and manufacturers, as described previously, are intended to be advisory and are not to be interpreted as approval of a request for an exception or alternative.

(Comment 3) One comment requests agreement from FDA that sponsors may administer multiple doses taken from individual preservative-free multi-dose vials in clinical trials prior to licensure, as long as the sponsor follows pre-approved aseptic procedures in defined time periods to support this format as part of the original license application.

(Response) FDA does not agree with the comment. The current regulation for preservatives requires that products in multiple-dose containers contain a preservative, with listed exceptions. The final rule provides the Director of CBER or CDER with flexibility to approve a request for an exception or alternative to this requirement. However, FDA will consider each request for an exception or alternative on a case-by-case basis and approval of such a request will be based on the determination that the data submitted with the request establishes a regulatory basis for approval. Sponsors seeking to investigate the use of a new biological product in humans must follow the procedures and requirements for investigational drugs under part 312. (See also Response to Comment 4).

(Comment 4) Several comments opposed the proposed rule because the commenters understood the rule to give the Director of CBER or CDER sole authority in the decisionmaking process to approve a request for an exception or alternative. Another comment stated that the proposed rule does not allow for a deliberative process for vaccine ingredient changes. Other comments stated that the drug industry had too much influence upon government agencies including FDA, and that all decisions about additives should reside with many experts, in order to avoid the potential of undue influence. One comment seeks greater transparency from FDA and manufacturers for all aspects of biologics. Another comment states that all changes to medicine, particularly those "which are proscribed by some government entities, should be subject to a public review."

(Response) FDA acknowledges and appreciates all comments on the proposed rule. FDA agrees with comments supporting public review and transparency. However, FDA disagrees with the comments opposing the authority of the Director of CBER or CDER to approve a biologic product. FDA also disagrees with the comments that the rule places the decisionmaking process in the hands of one person, does not allow for a deliberative process for vaccine ingredient changes, and that manufacturers will have an undue influence in the approval process.

Under the provisions of the PHS Act, and the Federal Food, Drug, and Cosmetic Act (the FD&C Act), FDA has the authority to issue and enforce regulations designed to ensure that biological products are safe, pure, and potent. Through delegations of authority,[8] the Directors of CBER and CDER have been given the authority to approve biological products. Thus, the Directors of CBER and CDER may approve a biologic product determined to be safe, pure, and potent, based on factors that include review of data, and in some cases, taking into account recommendations and input from independent experts (*e.g.,* advisory committees), input from interested parties, and public comments.

The PHS Act and the FD&C Act provide FDA with the authority to issue regulations that not only establish the requirements for product approvals but also establish the requirements for clinical investigations of unapproved biologics (21 U.S.C. 355(i) and 42 U.S.C. 262(a)(2)(A)). In accordance with part 312, manufacturers seeking to investigate the use of a new biological product in humans must follow specified procedures and requirements for investigational biological products. During the IND process, manufacturers must submit, for FDA review, data and proposals for additional studies intended to support the safety, purity, and potency of a biological product. Manufacturers also are required to provide information on patient outcomes and adverse events observed during this investigation. FDA reviews the submitted data and, upon determining that the biological product does not represent an unreasonable risk to the safety of the persons who are the subjects of the clinical investigation, will allow a manufacturer to proceed with the investigational use of a biological product. A manufacturer, after developing data to support approval, may submit a BLA to FDA for review and approval.

Under § 601.2, the Director of CBER or CDER may approve a manufacturer's application for a biologics license only after a manufacturer submits an application accompanied by data derived from nonclinical laboratory and clinical studies that demonstrate that the manufactured product meets requirements of safety, purity, and potency. These data are reviewed by appropriate experts to determine whether the application meets the statutory and regulatory requirements. In addition to the recommendations made by these experts, the Director of CBER or CDER may seek input from other sources within and outside of FDA to determine whether the application should be approved. Further, FDA closely monitors the safety of a biological product during its pre-approval and post-approval development, and may take corrective action, as necessary to protect the public.

In addition to the review process described previously, a sponsor, applicant, or manufacturer of a biological product regulated under the PHS Act (42 U.S.C. 262), may request review of a scientific controversy by an appropriate scientific advisory panel (§ 10.75(b)(2) (21 CFR 10.75(b)(2)). Also, under § 10.75(c), interested persons outside of FDA may request internal review of a decision through established FDA channels of supervision or review.

Thus, the current regulations establish procedures for review and evaluation of biological products, which include review by appropriate internal and external experts. In addition, the current regulations allow for public and private entities to participate in FDA's review process, as appropriate. This process serves to increase transparency and helps ensure that the public health is protected. The final rule maintains these important regulatory procedures and requirements while increasing FDA's flexibility in employing advances in science and technology.

(Comment 5) Several comments opposed the proposed rule because the commenters believe the rule would make the use of vaccines less safe. One commenter stated that FDA is ignoring its mandate to make vaccines safer by any and all means at its disposal; that FDA is making vaccines less safe by removing the certainty as to the minimum standards that a biological product must meet; and that the proposed rule does not require that the written requests for such exemptions or alternatives include the appropriate proofs (toxicological and immunological) of the short-term and long-term safety to the most susceptible humans. A few comments stated that an increase in the amount of aluminum may compromise the safety of vaccines. Another comment stated that families do not feel that the current regulations are "too prescriptive and unnecessarily restrictive," and that families would prefer more stringent rules. Other comments discussed specific concerns with already-approved vaccines.

(Response) FDA acknowledges these comments, as many of the issues were considered in drafting the proposed rule. However, FDA disagrees with the assertion that the rule will result in a decrease in the safety of vaccines and other biological products for which a request for an exception or alternative to any requirement under § 610.15 is made and approved. These regulations will continue to be the criteria by which all license applications will be evaluated. However, in order to employ advancements in treatment for certain populations, such as treatment for individuals suffering from life-threatening conditions (*e.g.,* cancer), FDA needs flexibility in applying the regulations. By analogy, as is stated in the drug regulations at 21 CFR 314.105(c):

> While the statutory standards apply to all drugs, the many kinds of drugs that are subject to statutory standards, and the wide range of uses for those drugs demand flexibility in applying the standards. Thus FDA is required to exercise its scientific judgment to determine the kind and quantity of data and information an applicant is

[8] Delegations of authority give certain officials in CBER and CDER the legal authority to take substantive actions and perform certain functions of the Commissioner of Food and Drugs. Staff Manual Guide 1410.702 available on the Internet at *http://www.fda.gov/AboutFDA/ReportsManualsForms/StaffManualGuides/ucm049563.htm* (accessed October 22, 2010); "Drug and Biological Product Consolidation," (68 FR 38067, June 26, 2003).

required to provide for a particular drug to meet the statutory standards.

The final rule is consistent with this CDER regulation as it allows the Directors of CBER and CDER flexibility in applying current standards for the approval of an exception or alternative to § 610.15, when data submitted with the request for an exception or alternative, establish the safety, purity, and potency of the biological product.

Further, consistent with existing statutory and regulatory requirements, the Directors of CBER and CDER will not approve a biological product that is unsafe for the intended population. The final rule does not alter these statutory and regulatory requirements nor does it guarantee that a request for an exception or alternative will be approved. The final rule only allows the Director of CBER or CDER the flexibility to approve a manufacturer's request for an exception or alternative if the manufacturer demonstrates that the biological product is safe, pure, and potent for use in the intended population.

With regard to comments expressing concern about the safety of previously licensed vaccines or specific ingredients in previously licensed vaccines, FDA notes that those comments concerning previously licensed vaccines are outside the scope of this rulemaking action because the rule only allows the Director of CBER or CDER to approve a manufacturer's request for an exception or alternative to any requirement in § 610.15, when the data submitted in support of such a request establish the safety, purity, and potency of the biological product.

(Comment 6) One comment opposed the proposed rule because the commenter did not know how FDA would monitor or enforce requirements for adequate storage, aseptic withdrawing techniques, and timely use of vaccines in multiple-dose containers without preservative or if additional training would be given to health care providers.

(Response) In addressing this comment, FDA clarifies that all requests for an exception or alternative are subject to FDA regulations regarding the monitoring and enforcement of regulatory standards. These regulations were established to assure the quality and integrity of data submitted to FDA in support of new product approvals and to protect the rights and welfare of the public. FDA accomplishes this through various means, including conducting onsite inspections, data audits, product testing, and report monitoring. FDA also provides advice through guidances and other communications which are provided to assist interested parties in complying with regulatory standards for the safety, purity, and potency of a product.

(Comment 7) One comment provided alternative revisions to the proposed rule and other subsections within § 610.15. Specifically, the commenter proposed that FDA revise the proposed rule to read as follows:

> Alternatives. Except for the generally accepted standards of purity and quality, in keeping with the vaccine safening mandates set forth in 42 U.S.C. 300aa–27"; * * * "the Director of the Center for Biologics Evaluation and Research or the Director of the Center for Drug Evaluation and Research may approve an exception or alternative to any requirement in this section, provided the manufacturer proves that the exception or alternative would improve the safety of the biological drug product or, failing that, improves the effectiveness, not efficacy, or reduces the per dose cost, of the biological drug product without reducing the safety of said product"; and * * * "include the findings, pro and con, of and the data from all of the studies conducted to support the request.

(Response) FDA acknowledges the comment and appreciates the suggestions for revising § 610.15. However, in accordance with the regulations, FDA is seeking public comment only on the proposed rule to permit the Director of CBER or the Director of CDER, as appropriate, to approve exceptions or alternatives to the regulation for constituent materials. FDA's response to the comments requesting revisions to the proposed rule are discussed in the paragraphs that follow.

FDA disagrees with the commenter's suggested revisions to the proposed rule because the revisions inappropriately limit the application of the rule to vaccines; allow more flexibility than is intended for approving a manufacturer's request for an exception or alternative; may lead to confusion about the rule; and are unnecessary. As discussed previously, the final rule allows the Director of CBER or CDER flexibility to approve a request for an exception or alternative to a requirement under § 610.15 provided that data are submitted that establish the safety, purity, and potency of the specific biological product. These statutory and regulatory requirements apply to the use of constituent materials in all biological products and not just to vaccines as the comment suggests. In addition, FDA may only approve a BLA for a vaccine or other biological product if it has been demonstrated to be "safe, pure, and potent." The commenter's suggestions that FDA should take cost considerations into account when making a decision to approve a vaccine are inconsistent with FDA's regulatory authority. Although FDA is sensitive to issues of cost, current statutory standards for constituent materials are based on the safety, purity, and potency of the product. Furthermore, the suggested revisions to the proposed rule inappropriately limit what FDA may consider with respect to a request for an exception or alternative. Manufacturers are required by current regulations to submit all available data, including adverse event reports, with a BLA. FDA reviews the data to determine whether an application should be approved. The final rule, as consistent with current regulations, does not allow the Director of CBER or CDER to approve an application if the data are not sufficient to establish that the biological product is safe, pure, and potent in relation to the manufacturer's intended use of the product.

IV. Legal Authority

FDA is issuing this regulation under the biological products provisions of the PHS Act (42 U.S.C. 262 and 264) and the drugs and general administrative provisions of the FD&C Act (sections 201, 301, 501, 502, 503, 505, 510, 701, and 704) (21 U.S.C. 321, 331, 351, 352, 353, 355, 360, 371, and 374). Under these provisions of the PHS Act and the FD&C Act, we have the authority to issue and enforce regulations designed to ensure that biological products are safe, pure, and potent; and prevent the introduction, transmission, and spread of communicable disease.

V. Analysis of Impacts

A. Review Under Executive Order 12866, the Regulatory Flexibility Act, and the Unfunded Mandates Reform Act of 1995

FDA has examined the impacts of the final rule under Executive Order 12866 and the Regulatory Flexibility Act (5 U.S.C. 601–612), and the Unfunded Mandates Reform Act of 1995 (Pub. L. 104–4). Executive Order 12866 directs agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). The Agency believes that this final rule is not a significant regulatory action under the Executive order.

The Regulatory Flexibility Act requires agencies to analyze regulatory

options that would minimize any significant impact of a rule on small entities. Because the final rule allows the Director of CBER or the Director of CDER, as appropriate, to approve exceptions or alternatives to the regulations for constituent materials, this action increases the flexibility and reduces the regulatory burden for affected entities. Therefore, FDA certifies that the final rule will not have a significant economic impact on a substantial number of small entities.

Section 202(a) of the Unfunded Mandates Reform Act of 1995 requires that agencies prepare a written statement, which includes an assessment of anticipated costs and benefits, before proposing "any rule that includes any Federal mandate that may result in the expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100,000,000 or more (adjusted annually for inflation) in any one year." The current threshold after adjustment for inflation is $135 million, using the most current (2009) Implicit Price Deflator for the Gross Domestic Product. FDA does not expect this final rule to result in any 1-year expenditure that would meet or exceed this amount.

The benefit of this regulatory action is its reduction, through greater flexibility in the regulatory requirements, of burdens on the biological products industry. These issues are discussed in greater detail in section I of this document. Industry cost reductions may result in consumers being offered lower prices or wider availability of existing and new biological products; this would have a positive effect on patients' welfare.

Any administrative and paperwork costs associated with this regulatory action are expected to be minimal and widely dispersed among affected entities. Based on FDA experience, we estimate that we would receive a total of approximately three requests annually for an exception or alternative under § 610.15. FDA experience with similar information collection requirements suggests that approximately 1 hour would be required to prepare and submit each such request.

We received comments expressing concern that this rule would generate additional costs in the form of negative public health effects. FDA has considered the potential for adverse consequences, including increased morbidity and mortality, associated with allowing deviations from the constituent materials regulations set forth in § 610.15(a) through (c), and will grant exemptions only in cases where data indicate that biological products in their exempted forms will be safe, pure, and potent for the conditions for which the applicant is seeking approval. As experience with the October 1981 rule has shown, FDA is able to conduct a constituent materials exemption process in a manner that is consistent with its public health mandate. For all these reasons, we believe the final rule will impose no overall public health cost.

B. Environmental Impact

The Agency has determined under 21 CFR 25.31(h) that this action is of a type that does not individually or cumulatively have a significant adverse effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

C. Federalism

FDA has analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the final rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, the Agency has concluded that the final rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

VI. Paperwork Reduction Act of 1995

Section 610.15(d) of this final rule contains reporting requirements that were submitted for review and approval to the Director of the Office of Management and Budget (OMB), as required by section 3507(d) of the Paperwork Reduction Act of 1995. The requirements were approved and assigned OMB control number 0910–0666.

List of Subjects in 21 CFR Part 610

Biologics, Labeling, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act and the Public Health Service Act, and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 610 is amended as follows:

PART 610—GENERAL BIOLOGICAL PRODUCTS STANDARDS

■ 1. The authority citation for 21 CFR part 610 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 360, 360c, 360d, 360h, 360i, 371, 372, 374, 381; 42 U.S.C. 216, 262, 263, 263a, 264.

■ 2. Amend § 610.15 by adding paragraph (d) to read as follows:

§ 610.15 Constituent materials.

* * * * *

(d) The Director of the Center for Biologics Evaluation and Research or the Director of the Center for Drug Evaluation and Research may approve an exception or alternative to any requirement in this section. Requests for such exceptions or alternatives must be in writing.

Dated: April 7, 2011.

Leslie Kux,

Acting Assistant Commissioner for Policy.

[FR Doc. 2011–8885 Filed 4–12–11; 8:45 am]

BILLING CODE 4160–01–P

DEPARTMENT OF JUSTICE

Drug Enforcement Administration

21 CFR Part 1314

[Docket No. DEA–347I]

RIN 1117–AB30

Self-Certification and Employee Training of Mail-Order Distributors of Scheduled Listed Chemical Products

AGENCY: Drug Enforcement Administration (DEA), Department of Justice.

ACTION: Interim final rule with request for comment.

SUMMARY: On October 12, 2010, the President signed the Combat Methamphetamine Enhancement Act of 2010 (MEA). It establishes new requirements for mail-order distributors of scheduled listed chemical products. Mail-order distributors must now self-certify to DEA in order to sell scheduled listed chemical products at retail. Sales at retail are those sales intended for personal use; mail-order distributors that sell scheduled listed chemical products not intended for personal use, *e.g.,* sale to a university, are not affected by the new law. This self-certification must include a statement that the mail-order distributor understands each of the requirements that apply under part 1314 and agrees to comply with these requirements. Additionally, mail-order distributors are now required to train their employees prior to self certification. DEA is promulgating this rule to incorporate the statutory provisions and make its regulations consistent with the new requirements

EXHIBIT 360

FEDERAL REGISTER

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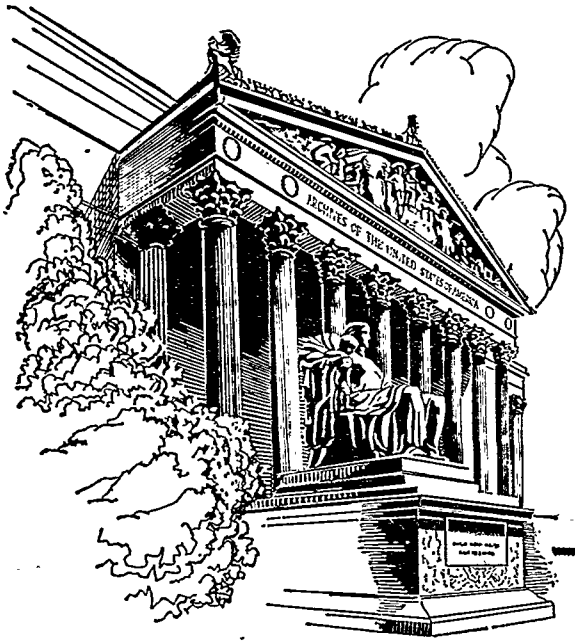

Exhibit 360

(r) Minimum potency of product expressed in terms of official standard of potency or, if potency is a factor and no U.S. standard of potency has been prescribed, the words "No U.S. standard of potency."

§ 73.52 Proper name; package label; legible type.

(a) *Position.* The proper name of the product on the package label shall be placed above any trade-mark or trade name identifying the product and symmetrically arranged with respect to other printing on the label.

(b) *Prominence.* The point size and type-face of the proper name shall be at least as prominent as the point size and type-face used in designating the trademark and trade name. The contrast in color value between the proper name and the background shall be at least as great as the color value between the trade-mark and trade name and the background. Typography, layout, contrast, and other printing features shall not be used in a manner that will affect adversely the prominence of the proper name.

(c) *Legible type.* All items required to be on the container label and package label shall be in legible type. "Legible type" is type of a size and character which can be read with ease when held in a good light and with normal vision.

23. Amend § 73.53 to read as follows:

§ 73.53 Divided manufacturing responsibility to be shown.

If two or more establishments participate in the manufacture of a product, the name, address, and license number of each must appear on the package label, and on the label of the container if capable of bearing a full label.

§ 73.73 [Amended]

24. Amend § 73.73(d)(1) by deleting the word "container".

25. Amend § 73.73(d)(2) by changing "container" to "vessel" after the word "bulk" at the end of the first sentence.

26. Amend § 73.73(e)(2)(i) by changing "container" to "test vessel" in the title and by changing "container" to "vessel" in the text.

27. Amend § 73.78 to read as follows:

§ 73.78 Constituent materials.

(a) *Ingredients, preservative, diluents, adjuvants.* All ingredients used in a licensed product, and any diluent provided as an aid in the administration of the product, shall meet generally accepted standards of purity and quality. Any preservative used shall be sufficiently nontoxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in the combination used shall not denature the specific substances in the product below the minimum acceptable potency within the dating period when stored at the recommended temperature. Products in multiple dose containers shall contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine, Poliovirus Vaccine, Live, Oral, or to viral vaccines labeled for use with the jet injector, or to dried vaccines when the accompanying diluent contains a preservative. An adjuvant shall not be affect adversely the safety or potency of product contain more than 0.85 milligram of aluminum, determined by assay, or more than 1.14 milligrams of aluminum, determined by calculation on the basis of the amount of aluminum compound added.

(b) *Extraneous protein; cell culture produced vaccines.* Extraneous protein known to be capable of producing allergenic effects in human subjects shall not be added to a final virus medium of cell culture produced vaccines intended for injection. If serum is used at any stage, its calculated concentration in the final medium shall not exceed 1:1,000,000.

(c) *Antibiotics.* A minimum concentration of antibiotics, other than penicillin, may be added to the production substrate of viral vaccines.

28. Amend § 73.83 by revising the first sentence to read as follows:

§ 73.83 Date of manufacture.

The date of manufacture shall be determined as follows:

* * * * *

29. Amend § 73.84 to read as follows:

§ 73.84 Periods of cold storage.

Except as otherwise provided in the regulations of this part, products may be held in cold storage by the manufacturer as follows:

At a temperature not above 5°C.—1 year.
At a temperature not above 0°C.—2 years.

30. Amend § 73.85 to read as follows:

§ 73.85 Dating period.

The dating period for a combination of two or more products shall be no longer than the dating period of the component product with the shortest dating period. The dating period for a product shall begin on the date of manufacture, except that the dating period may begin on the date of issue from the manufacturer's cold storage, provided the product was maintained as prescribed in § 73.84. If held in the manufacturer's cold storage beyond the period prescribed, the dating period shall be reduced by a corresponding period.

31. Amend § 73.144(a).

32. Paragraph (b) of § 73.144 is deleted.

33. Paragraph (c) of § 73.144 is deleted.

34. Amend § 73.144(e).

35. Amend § 73.144(h).

The affected portions of § 73.144 read as follows:

§ 73.144 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 73.75, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b) [Deleted]

(c) [Deleted]

* * * * *

(e) *Labeling.* In addition to the item required by other applicable labeling provisions of this part, single-dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

* * * * *

(h) *Samples and protocols.* For each lot of vaccine, the following materials shall be submitted to the Director, Division of Biologics Standards, National Institutes of Health, Bethesda, Md. 20014:

(1) A protocol which consists of a summary of the history of the manufacture of each lot including all results of each test for which test results are requested by the Director, Division of Biologics Standards.

(2) A total of no less than 120 ml. in 10 ml. volumes, in a frozen state (—60° C.), of preclarification bulk vaccine containing no preservative or adjuvant, and no less than 100 ml. in 10 ml. volumes, in a frozen state (—60° C.), of post-clarification bulk vaccine containing stabilizer but no preservative or adjuvant, taken prior to filling into final containers.

(3) A total of no less than 200 recommended doses of the vaccine in final labeled containers distributed equally between the number of fillings made from each bulk lot, except that the representation of a single filling shall be no less than 30 final containers.

36. Amend the first sentence of § 73.151(c) to read as follows:

§ 73.151 Manufacture of Measles Virus Vaccine, Inactivated.

* * * * *

(c) *Virus propagated in monkey kidney tissue cultures.* Only Macaca or Cercopithecus monkeys, or a species found by the Director, Division of Biologics Standards, to be equally suitable, which have met all the quarantine requirements, shall be used as the source of kidney tissue for the manufacture of Measles Virus Vaccine, Inactivated.

* * * * *

37. Section 73.154(a) is deleted.

38. Amend § 73.154(b).

39. Section 73.154(d) is deleted.

40. Section 73.154(e) is deleted.

41. Amend § 73.154(f)(4).

The affected portions of § 73.154 read as follows:

§ 73.154 General requirements.

(a) [Deleted]

(b) *Extraneous protein.* The final vaccine shall have a protein nitrogen content of less than 0.02 milligram per individual human dose.

* * * * *

(d) [Deleted]

(e) [Deleted]

(f) * * *

(4) A protocol which consists of a summary of the history of the manufacture of each lot including all results of

EXHIBIT 361

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In vivo absorption of aluminium-containing vaccine adjuvants using ^{26}Al

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Aluminium hydroxide (AH) and aluminium phosphate (AP) adjuvants, labelled with ^{26}Al, were injected intramuscularly (i.m.) in New Zealand White rabbits. Blood and urine samples were collected for 28 days and analysed for ^{26}Al using accelerator mass spectrometry to determine the absorption and elimination of AH and AP adjuvants. ^{26}Al was present in the first blood sample (1 h) for both adjuvants. The area under the blood level curve for 28 days indicates that three times more aluminium was absorbed from AP adjuvant than AH adjuvant. The distribution profile of aluminium to tissues was the same for both adjuvants (kidney > spleen > liver > heart > lymph node > brain). This study has demonstrated that in vivo *mechanisms are available to eliminate aluminium-containing adjuvants after i.m. administration. In addition, the pharmacokinetic profiles of AH and AP adjuvants are different. © 1997 Elsevier Science Ltd.*

Keywords: adjuvant absorption, antigen desorption, ^{26}Al

Vaccines usually contain an antigen and an adjuvant, which potentiates the immune response to the antigen. The adjuvant effect of aluminium-containing compounds was first observed in 1926[1]. Since that time aluminium hydroxide adjuvant and aluminium phosphate adjuvant have been widely used in both human and animal vaccines. These are the only adjuvants that are currently approved for use in human vaccines by the United States Food and Drug Administration (FDA).

A recent study[2] has shown that aluminium hydroxide (AH) adjuvant is crystalline aluminium oxyhydroxide, AlOOH. It has a fibrous morphology and dissolves very slowly in simulated interstitial fluid[3]. Aluminium phosphate (AP) adjuvant is amorphous aluminium hydroxyphosphate. It has a platy morphology and dissolves more rapidly in simulated interstitial fluid than AH adjuvant. Interstitial fluid contains three organic acids which have an α-hydroxy carboxylic acid group (citric, lactic and malic acids), and are therefore capable of chelating aluminium[4–6]. A recent *in vitro* study[3] showed that citrate anion was able to dissolve both AH and AP adjuvants, although AP adjuvant dissolved more rapidly.

Vaccines containing AH or AP adjuvants are usually administered intramuscularly. The FDA limits the quantity of the adjuvant to no > 0.85 mg aluminium per dose. The disposition of aluminium-containing adjuvants after intramuscular (i.m.) administration is not understood. This is largely because the low dose of aluminium does not cause detectable changes in the concentration of aluminium normally present in blood, urine or tissues. Measurement of ^{26}Al by accelerator mass spectrometry (AMS)[7,8] offers the first opportunity to directly determine if aluminium-containing adjuvants are removed from the site of injection by dissolution in interstitial fluid. In addition, AMS allows the absorption, distribution and elimination profiles of aluminium-containing adjuvants to be studied and optimized.

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MATERIALS AND METHODS

Adjuvants

^{26}Al-containing AH adjuvant was prepared by adding 0.596 g of an $^{26}AlCl_3$ solution in 0.1 N HCl (170 Bq $^{26}Al\ g^{-1}$ or 0.24 μg $^{26}Al\ g^{-1}$) to 45 ml of 0.2 M $AlCl_3$. Forty-five milliliters of a 0.6 N NaOH and 4 M NaCl solution was added dropwise over 30 min to the $AlCl_3$/$^{26}AlCl_3$ solution with vigorous agitation. The precipitate was repeatedly washed with 50 ml portions of double distilled water (ddH_2O) after centrifugation until the supernatant was free of chloride as determined by the absence of a precipitate when 0.1 M $AgNO_3$ was

added. The washed precipitate was resuspended in 50 ml of ddH_2O, filled into a sealed container and placed in an 80°C oven for 24 h. After heating, the volume was adjusted to 57.1 ml with ddH_2O. The adjuvant suspension was autoclaved at 121°C for 20 min. A dose of 0.20 ml contains 0.85 mg Al. The preceding procedure without the $^{26}AlCl_3$ was followed to produce an AH adjuvant for testing. The tests showed that the AH adjuvant prepared by this procedure exhibited the X-ray diffraction pattern and infrared spectrum which are typical of AH adjuvant[2].

^{26}Al-containing AP adjuvant was prepared by dissolving 3.7 g of alum [$KAl(SO_4)_2 \cdot 12\ H_2O$] in enough ddH_2O to make 68 ml and adding 0.519 g of the $^{26}AlCl_3$ solution in 0.1 N HCl (170 Bq $^{26}Al\ g^{-1}$ or 0.24 μg $^{26}Al\ g^{-1}$). A phosphate solution was prepared (0.3403 g $NaH_2PO_4 \cdot H_2O$, 0.3501 g Na_2HPO_4 and 5.5796 g NaCl) in enough ddH_2O to make 800 ml. The alum solution was slowly added to the phosphate solution and agitated until the solution was clear. The solution was titrated with 1N NaOH with agitation until the pH was 7.1–7.2 to precipitate aluminium hydroxyphosphate. The suspension was agitated for 2 h and the pH readjusted to 7.1–7.2 with 1 N NaOH. The precipitate was washed three times with 0.9% NaCl by centrifugation. After the third wash, the sediment was dispersed in enough 0.9% NaCl to make 50 ml. The adjuvant suspension was autoclaved at 121°C for 20 min. A dose of 0.20 ml contains 0.85 mg Al. The preceding procedure without the $^{26}AlCl_3$ was followed to produce an AP adjuvant for testing. The tests showed that the AP adjuvant prepared by this procedure was amorphous by X-ray diffraction and the infrared spectrum was typical of AP adjuvant[2].

^{26}Al-containing aluminium citrate was prepared by dissolving 0.7606 g $AlCl_3 \cdot 6\ H_2O$ in enough ddH_2O to make 10 ml. Twenty-one microliters of the $^{26}AlCl_3$ solution in 0.1 N HCl (170 Bq $^{26}Al\ g^{-1}$ or 0.24 μg $^{26}Al\ g^{-1}$) was added with mixing. A citric acid solution was prepared by dissolving 0.6620 g of citric acid in enough ddH_2O to make 10 ml. The citric acid solution was added to the $AlCl_3/^{26}AlCl_3$ solution and mixed. The pH was adjusted to 7.4 with 0.1 N NaOH.

The specific activity of the ^{26}Al-labelled adjuvants was 15.9 Bq ml^{-1} for the AH adjuvant and 15.5 Bq ml^{-1} for the AP adjuvant. The specific activity of the ^{26}Al-labelled aluminium citrate solution was 1.07 Bq ml^{-1}. Thus, the doses contained 3.2 Bq for the AH adjuvant (i.m.), 3.1 Bq for the AP adjuvant (i.m.) and 0.32 Bq for the aluminium citrate solution (intravenous; i.v.). Calibration errors were 3–5%.

Rabbits

Six female New Zealand White rabbits were used to determine the *in vivo* absorption of the ^{26}Al-labelled adjuvants. They were conditioned for 21 days before the study and their weights were 2.5–2.8 kg at the beginning of the study and 3.2–3.7 kg at the end of the study.

Two rabbits received an i.m. injection (0.2 ml of ^{26}Al-labelled adjuvant followed by 0.1 ml of sterile 0.9% NaCl to wash the syringe) of ^{26}Al-labelled AH adjuvant, two rabbits received a similar i.m. injection of ^{26}Al-labelled AP adjuvant, one rabbit received an equivalent i.v. injection (0.3 ml of ^{26}Al-labelled aluminium citrate followed by 0.1 ml of sterile 0.9% NaCl to wash the syringe) of ^{26}Al-labelled aluminium citrate, and one rabbit received an equivalent i.m. dose of AP adjuvant containing no ^{26}Al as a cross-contamination monitor. All rabbits received a total of 0.85 mg aluminium.

The rabbits were killed 28 days after the injections by sodium pentobarbital overdose. This study was approved by the Purdue University Animal Care and Use Committee and performed in accordance with all federal regulations.

Sample collection

One milliliter of whole blood was collected at 0, 1, 2, 4, 6, 10, and 12 h and at 1, 2, 4, 6, 8, 12, 16 and 21 days. Three milliliters of blood were collected at 28 days. The samples were collected in 3 ml vials with premeasured ethylenediaminetetra-acetic acid and refrigerated immediately.

Urine was collected for 24 h before dosing and for the following intervals: 0–5, 5–9 and 9–24 h, 1–2, 2–4, 4–6, 6–8, 11–12, 15–16, 20–21 and 27–28 days. Urine was collected in screened pans placed under the cages. The pans were filled with 2 l of water at the beginning of each collection period. At the end of the collecting period, the pans were agitated and 40 ml aliquots were placed in 50 ml polypropylene centrifuge tubes and immediately refrigerated. The total volume of liquid in the pans when the aliquot was collected was recorded.

Tissue samples were collected after the rabbits were killed on day 28. Whole brain, heart, left kidney, liver, mesenteric lymph node and spleen tissues were collected and frozen in comercial plastic freezer bags. Bone (femur) samples were also collected, but these samples were lost during chemical preparation. The brain sample for one of the AP-dosed rabbits was also lost during chemical preparation.

Sample preparation

Blood and urine samples were prepared for AMS analysis by the addition of 1–100 mg ^{27}Al carrier from Al_3Cl (ICP 10000 p.p.m. ^{27}Al standard). The samples were then repeatedly digested in nitric acid (70%) at 80°C in a porcelain crucible and allowed to evaporate to dryness. After two digestions in nitric acid, the samples were ashed at 800°C to yield Al_2O_3 powder. This Al_2O_3 powder was then mixed with silver powder in a 1:3 ratio by mass and analysed by AMS.

Tissues were prepared by first dissolving the tissue in 20–200 ml (depending on tissue size) of nitric acid (70%) in polyethylene bottles. Aliquots of the dissolved tissue were then prepared as described above except that hydrogen peroxide (30%) was used as well as nitric acid in the wet digestion.

Data analysis

Since AMS measures relative amounts of ^{26}Al and ^{27}Al in samples, the actual recovery percentage of aluminium during sample preparation is irrelevant provided that the carrier ^{27}Al is homogenized with the ^{26}Al native to the sample. In order to test the reproducibility of the carrier addition, sample digestion, and AMS analyses, ten samples were separately prepared in triplicate. The results for each of these samples agreed

within 10% (standard error of the mean) or within the AMS precision.

Cross-contamination of ^{26}Al between the animals was monitored by the measurement of samples from the rabbit receiving no ^{26}Al dose. Data was rejected if the ^{26}Al concentration in a given sample was not at least five times higher than the equivalent sample from the cross-contamination monitor. Also, the ^{26}Al concentration in blood, urine and tissue samples from the cross-contamination monitor rabbit was subtracted from the ^{26}Al concentration in equivalent samples of the other rabbits.

Cross-contamination of ^{26}Al between samples during chemical preparation was monitored with the preparation of chemistry blanks. In no case did these blanks indicate more than a 1% cross-contamination during chemical preparation. Chemistry blanks are samples that are prepared alongside experimental samples. These blanks undergo the same preparation procedure in order to monitor any possible cross-contaminatin of ^{26}Al between samples during the chemical preparation of experimental samples.

All AMS analyses were conducted at the Purdue Rare Isotope Measurement Laboratory, PRIME Lab[9]. Although all samples were analysed for ^{26}Al content, data is reported in terms of aluminium arising from the ^{26}Al-labelled adjuvants or ^{26}Al-labelled aluminium citrate. The result for the 4 h blood sample for rabbit 1 was rejected and not included in any analysis due to an error in the recording of data for that sample.

RESULTS

Figure 1 shows the time profile for the aluminium blood concentration of the four rabbits receiving the ^{26}Al-labelled adjuvants. The blood level curve of both adjuvants exhibit an absorption phase and an elimination phase, as is typical of i.m. administration. It is noteworthy that ^{26}Al was found in the blood at the first sampling point (1 h) for both adjuvants. Thus dissolution of the adjuvants in interstitial fluid begins upon administration. The aluminium concentration produced by AH adjuvant at 1 h was similar to the concentrations found from 2 to 28 days.

The mean area under the blood concentration versus time curve (AUC) from days 0 to 28, determined using the trapezoid rule, was 1.6×10^{-3} mg h g^{-1} for the i.v. dose of ^{26}Al-labelled aluminium citrate ($n = 1$); 8.1×10^{-4} mg h g^{-1} for the ^{26}Al-labelled AP adjuvant ($n = 2$); and 2.7×10^{-4} mg h g^{-1} for the ^{26}Al-labelled AH adjuvant ($n = 2$). Thus, three times as much aluminium was absorbed from the AP adjuvant as from the AH adjuvant within 28 days. However, during the first 48 h (*Figure 1* insert), the AUC of the AH adjuvant was 1.4 times the AUC of the AP adjuvant. These data also indicate that 17% of the AH adjuvant and 51% of the AP adjuvant were absorbed within 28 days based on the AUC of the i.v. dose of ^{26}Al-labelled aluminium citrate. The blood concentration of aluminium for each of the rabbits receiving an adjuvant had not reached a terminal elimination phase by day 28.

Cumulative urinary excretion of aluminium (*Figure 2*) indicates that the body is able to eliminate the aluminium absorbed from the adjuvants. The cumulative amount of aluminium eliminated in the urine during the 28 days of the study was 6% of the AH adjuvant dose and 22% of the AP adjuvant dose. Aluminium from both adjuvants was still being excreted at a steady rate at day 28.

The pharmacokinetic parameters determined from the blood and urine data are presented in *Table 1*.

Distribution of aluminium in tissues 28 days after administration of AH and AP adjuvants is shown in *Figure 3*. For each tissue, the concentration of aluminium was greater in the rabbits which received AP adjuvant. The average aluminium tissue concentration was 2.9 times greater for AP adjuvant than for AH adjuvant.

DISCUSSION

It is noteworthy that the aluminium concentration produced by AH adjuvant at the first sampling point

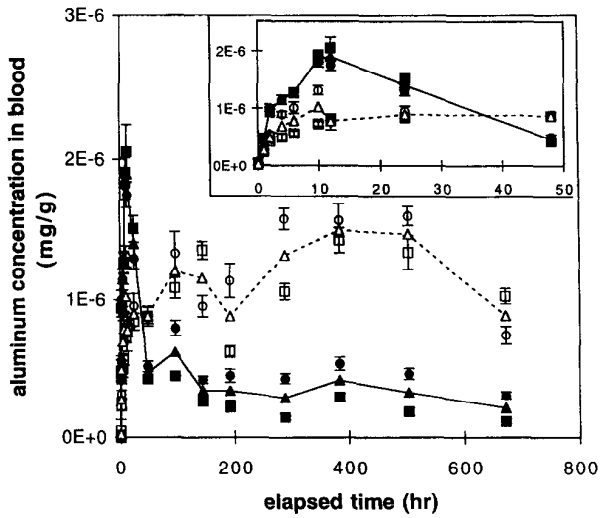

Figure 1 Blood concentration profile after i.m. administration of ^{26}Al-labelled aluminium hydroxide adjuvant: ■, rabbit 1; ●, rabbit 2; ▲, mean; or aluminium phosphate adjuvant: □, rabbit 3; ○, rabbit 4; △, mean

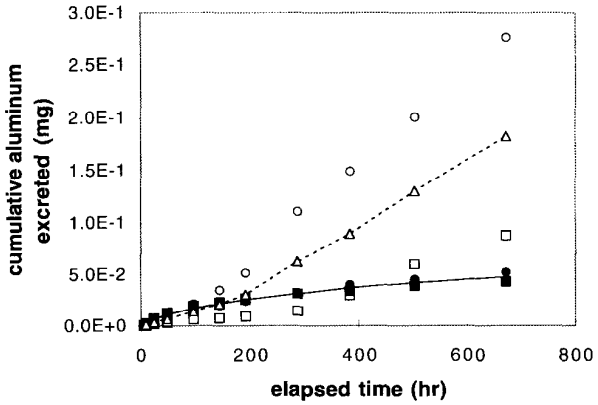

Figure 2 Cumulative urinary excretion of aluminium after i.m. administration of ^{26}Al-labelled aluminium hydroxide adjuvant: ■, rabbit 1; ●, rabbit 2; ▲, mean; or aluminium phosphate adjuvant: □, rabbit 3; ○, rabbit 4; △, mean. Error bars of <5% are not shown

(1 h) was similar to the 2–28 day concentrations. This indicates that dissolution of aluminium-containing adjuvants in interstitial fluid begins quickly after i.m. administration. It is surprising that the aluminium concentrations were greater during the first 24 h for crystalline AH adjuvant than for the amorphous AP adjuvant. This suggests that the initial rate of dissolution from the edges of the fibrous AH adjuvant particles is greater than from the platy AP adjuvant particles.

The rapid appearance of aluminium in the blood may have implications for theories regarding the mechanism of adjuvant action of aluminium-containing adjuvants. The most widely accepted theory is the repository effect[10], whereby the antigen adsorbed by the aluminium-containing adjuvant is slowly released after i.m. administration. The rapid appearance of aluminium as seen in the insert of *Figure 1* challenges the repository mechanism as it is likely that the adsorbed antigen would be quickly desorbed as a result of the fast initial dissolution of the substrate.

After 2 days, the absorption rate for AP adjuvant was considerably more than the AH adjuvant which confirms the difference in *in vitro* dissolution rates in simulated interstitial fluid[3]. The blood concentration of aluminium was fairly steady from days 2 to 28 indicating a relatively constant absorption rate for each adjuvant even 28 days after i.m. administration. No terminal phase had been reached for the blood concentration of aluminium so it is difficult to determine the mean residence time of each adjuvant, It is clear, however, that AP adjuvant will be eliminated before AH adjuvant because the long term absorption rate of the AP adjuvant is greater.

The measured increase in the plasma concentration of aluminium from the i.v. dose was *ca* 600 ng ml^{-1}, which is considerably more than the increase of 2 ng ml^{-1} from the i.m. dose. Since it has been shown that the pharmacokinetics of aluminium depend on the concentration in the blood[11], the pharmacokinetics of the i.v. bolus dose were probably somewhat different from those of the i.m. dose. Thus the AUC from the i.v. dose may not provide a completely accurate baseline for determining the fraction of the aluminium absorbed from the i.m. administration of the AH and AP adjuvants. However, this does not affect the relative comparison of the AH and AP adjuvants.

The two rabbits which received AH adjuvant exhibited very similar pharmacokinetic characteristics. The blood level data for the two rabbits receiving AP adjuvant were also very similar. However, the cumulative urinary excretion of aluminium differed by a factor of three between the two rabbits which received AP adjuvant. This difference is probably due to intersubject variability in the elimination of aluminium[12]. In spite of this intersubject variation, the cumulative urinary excretion of aluminium after 28 days in each rabbit receiving AP adjuvant was greater than the cumulative urinary excretion of aluminium in the rabbits receiving AH adjuvant.

The normal plasma aluminium concentration in rabbits is 30 ng ml^{-1}[13]. The maximum increase in the plasma aluminium concentration from the 0.85 mg aluminium doses of either adjuvant was *ca* 2 ng ml^{-1}. This small increase would have been masked by the aluminium background if ^{26}Al-labelled adjuvants were not used. If the same dose of these adjuvants was administered i.m. to adult humans, an increase in the plasma aluminium concentration of *ca* 0.04 ng ml^{-1}

Table 1 Pharmacokinetic parameters after i.m. injection of ^{26}Al-containing aluminium hydroxide and aluminium phosphate adjuvants

Adjuvant	AUC for 0–28 days (mg h g^{-1})	% Absorbed in 28 days	Cumulative aluminium in urine after 28 days (%)
Aluminium hydroxide			
Rabbit 1	2.0×10^{-4}	13	5.0
Rabbit 2	3.5×10^{-4}	22	6.2
Average	2.7×10^{-4}	17	5.6
Aluminium phosphate			
Rabbit 3	2.7×10^{-4}	47	10
Rabbit 4	8.7×10^{-4}	55	33
Average	8.1×10^{-4}	51	22

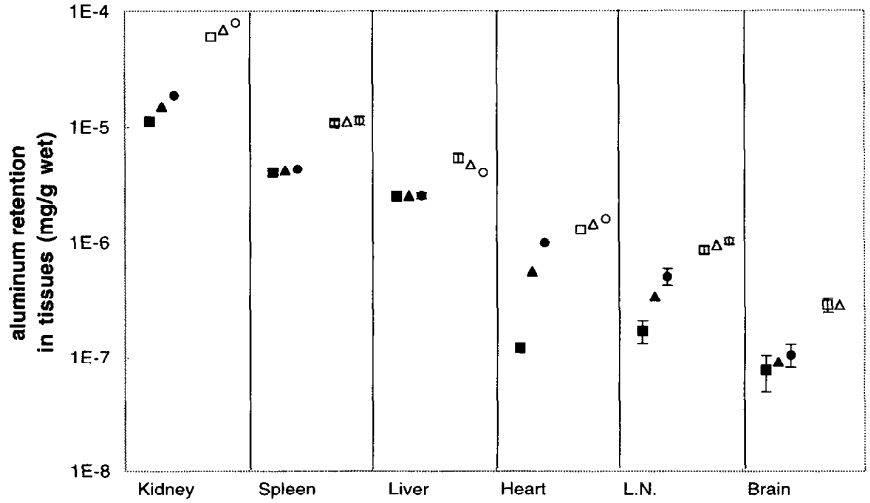

Figure 3 Aluminium tissue concentration 28 days after administration of ^{26}Al-labelled aluminium hydroxide adjuvant: ■, rabbit 1; ●, rabbit 2; ▲, mean; or aluminium phosphate adjuvant: □, rabbit 3; ○, rabbit 4; △, mean. L.N., lymph node. Error bars of <5% are not shown

could be expected based on the larger blood volume of humans and assuming the same rate of dissolution in interstitial fluid. This represents a 0.8% increase in plasma aluminium concentration based on a normal value of 5 ng ml [14]. This small change explains the safety of aluminium-containing adjuvants and emphasizes the utility of AMS for studying aluminium concentration *in vivo*.

The relative tissue distribution was the same for both adjuvants (kidney > spleen > liver > heart > lymph node > brain). This distribution pattern is typical of results obtained when ^{26}Al was given by other routes of administration[15]. Since the concentration of aluminium was 2.9 times greater on average in each tissue (*Figure 3*) for the rabbits which received AP adjuvant, the tissue data is consistent with the ratio of 3.0 which was observed for the AUC of AP adjuvant compared to AH adjuvant. Thus, the relative ^{26}Al tissue concentrations can be inferred from the ^{26}Al blood concentrations.

Since the adjuvants are being dissolved by interstitial fluid which flows directly into the lymphatic system, one may expect the aluminium concentration to be quite high in the lymph tissue that was collected. However, the i.m. doses were given in the hind quarter where the nearest lymph node is difficult to isolate. For this reason, the mesenteric lymph node, located in the abdominal cavity, was removed. Thus the aluminium from the dissolved adjuvants does not flow directly to the lymph tissue that was collected and measured.

Dissolution, absorption, distribution and elimination of aluminium-containing adjuvants after i.m. administration has been demonstrated by the use of ^{26}Al-labelled adjuvants. The two adjuvants studied exhibited significantly different dissolution rates in interstitial fluid which were reflected in different blood, urinary excretion and tissue profiles. Human studies using ^{26}Al-labelled adjuvants can be performed since the radiation exposure to ^{26}Al is negligible. There was 1.6 Bq ^{26}Al used in each rabbit. In humans, *ca* 74 Bq ^{26}Al would need to be used resulting in a maximum whole body exposure to radiation of *ca* 15 μSv year^{-1} compared to the natural background exposure of 3000 μSv year^{-1}.

The application of AMS to the *in vivo* performance of vaccines should lead to a fuller understanding of the mechanism of adjuvant action of aluminium-containing adjuvants. The ability to label an aluminium-containing compound with ^{26}Al, as demonstrated in this study, may prove useful in studying the *in vivo* absorption, distribution, metabolism and elimination profiles of other aluminium-containing compounds.

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EXHIBIT 362

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Aluminium assay and evaluation of the local reaction at several time points after intramuscular administration of aluminium containing vaccines in the Cynomolgus monkey

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Abstract

Aluminium hydroxide and aluminium phosphate have been widely used as vaccine adjuvants with a good safety record for several decades. The recent observation in human deltoid muscle of macrophage aggregates containing aluminium hydroxide spicules and termed Macrophagic Myofasciitis (MMF) has encouraged research on aluminium salts. This study was conducted in order to further investigate the clearance of aluminium at the vaccine injection site and the features of induced histopathological lesions. Two groups of 12 monkeys were immunised in the quadriceps muscle with Diphtheria–Tetanus vaccines, which were adjuvanted with either aluminium hydroxide or aluminium phosphate. Three, six or twelve months after vaccination, four monkeys from each group were sacrificed and histopathological examination and aluminium assays were performed on quadriceps muscle sections.

Histopathological lesions, similar to the MMF described in humans, were observed and were still present 3 months after aluminium phosphate and 12 months after aluminium hydroxide adjuvanted vaccine administration. An increase in aluminium concentration, more marked in the area of the lesions, was also observed at the 3- and 6-month time points. These findings were localised at the injection site and no similar changes were observed in the distal or proximal muscle fragments.

We conclude from this study that aluminium adjuvanted vaccines administered by the intramuscular route trigger histopathological changes restricted to the area around the injection site which persist for several months but are not associated with abnormal clinical signs.

Keywords: Aluminium; Macrophagic myofasciitis; Animal study

1. Introduction

Aluminium salts have been used as vaccine adjuvants since the initial proof of concept in an animal model by Glenny et al. [1] in 1926. This type of metal salt remains the only class of adjuvant accepted in a wide range of vaccines such as Tetanus, Diphtheria, Pertussis, Hepatitis A and Hepatitis B [2]. The unique exception to this broad use of aluminium is a lipid-based adjuvant, MF 59, the adjuvant used in a European flu vaccine.

There are several potential mechanisms for the mode of action of aluminium adjuvant [3] which are still being investigated [4,5]. These mechanisms are as follows: (a) depot formation allowing a slow release of the antigen, (b) arrangement of the aluminium adjuvanted vaccine in a particulate form which is better processed by antigen presenting cells, and (c) stimulation of the immune system via an inflammatory reaction with the release of immune mediators.

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Due to the extensive use of this adjuvant, there is a large amount of data indicating its good safety profile. Some case studies reporting local reactions after administration of vaccines using aluminium as the adjuvant either by the subcutaneous or intramuscular routes have been published [6–10]. However, as aluminium has never been administered separately from the vaccine formulation, all data should always be considered to be related to the adjuvanted vaccine as a single entity, which is a mixture of one or several antigens plus the adjuvant [11]. Consequently definitive correlation of any of the reported findings to aluminium itself can be challenged. The metal aluminium, related assay methods, sources of human and environmental exposure, kinetics, metabolism and toxicity have also been studied in detail [12]. These findings state that aluminium is widely distributed in water, air, food, cosmetics and pharmaceuticals in relatively high concentrations. By comparison with natural or environmental exposure as stated in this review from the WHO [12], exposure to the very low quantity of aluminium administered as an adjuvant in a vaccine would not seem to raise major safety concerns.

Despite this reassuring comparison with natural exposure, French scientists (former GERMMAD) recently described a focal histological lesion observed in biopsy samples from the deltoid muscle of the non-dominant arm, which they termed Macrophagic Myofasciitis (MMF). These biopsies were conducted following patients' reports of clinical symptoms observed in muscular disorders, which generally combined persistent myalgias, arthralgias and marked fatigue [13]. Interestingly, there were no apparent links between the anatomical distribution of muscular weakness and the localised deltoid lesion. In these biopsies of the deltoid muscle aluminium hydroxide spicules [14] were identified in the macrophages of the lesions, potentially incriminating aluminium adjuvants in the aetiology of this local histopathological entity in the muscle [15]. However, due to the lack of appropriate controls and the very limited number of cases, the role of aluminium and the causal relationship between focal MMF in the deltoid muscle and a more widespread muscle weakness are still being disputed.

A WHO meeting [16] dedicated to this issue associated with aluminium, emphasised the need for more research on this topic. Direct investigation in humans is difficult; both the pain and the remaining scar associated with a muscular biopsy are barriers to studying potential local lesions in the injected muscles of vaccinated people. In addition, epidemiological studies are complicated by the fact that the occurrence of this set of reactions is very low (i.e., approximately 200 cases in several tens of millions of vaccinated people) and by the lack of a case definition. Therefore, non-clinical studies may usefully contribute to confirmation or invalidation of the potential association between aluminium salts and the local histological lesions, termed MMF, and also between local deposits of aluminium salts and generalised clinical symptoms. In addition preclinical studies may provide information on the distribution of aluminium following administration of adjuvanted vaccine. Distribution has been reported [17,18]; however, these studies lacked the sensitivity of detection possible with modern apparatus. Fortunately, Stanley Hem and his collaborators [19,20] recently addressed this issue and applied a radioactive method using ^{26}Al and accelerator mass spectrometry to compare the deposition following intramuscular administration of aluminium oxyhydroxide (AlOOH) and aluminium phosphate (AlPO4). However, this work did not address the question of clearance of aluminium from the site of injection in the muscle nor did they use a complete vaccine formulation including the adjuvant to test for aluminium deposition in conditions similar to those used in man.

Several critical questions remain:

- How long does the aluminium stay in the muscle after intramuscular administration of adjuvanted vaccines?
- Does aluminium adjuvanted vaccine "in essence" trigger a histological reaction, which can be termed MMF?
- If there is such a reaction what are its features (size, identification of the cells, persistence)
- Does such a local muscular lesion characterise a more widespread muscular disease?

This study was conducted to address these questions to a certain extent by the evaluation of the local reaction and aluminium concentration after intramuscular injection of aluminium adjuvanted vaccines in Cynomolgus monkeys.

2. Materials and methods

2.1. Vaccines

Combined Diphtheria–Tetanus vaccines were prepared by Aventis Pasteur with either AlOOH from Reheis (Ireland) or AlPO4 from Biosector (Denmark). The two Diphtheria–Tetanus vaccines, identical in all respects except for the aluminium salts, contained 30 Lf/ml of Diphtheria, 10 Lf/ml of Tetanus toxoid, and adjuvants (AlPO4 or AlOOH) corresponding to a final concentration of 0.6 mg/ml Al. These vaccines also both contained Merthiolate as a preservative.

2.2. Animal immunisation

Two groups of 12 male Cynomolgus monkeys (*Macaca fasciculata*) supplied by CRP le vallon (Mauritius), weighing 2.3–3.9 kg at the beginning of the study, were given a single intramuscular vaccine injection with a 10 mm needle carefully oriented perpendicular to the skin at the midshaft femoral area of the quadriceps muscle. Either AlPO4 adjuvanted DT or AlOOH adjuvanted DT vaccine (as detailed above) was administered at a dose volume of 0.5 ml per monkey. The injection site was identified by an ink tattoo on the skin to increase the precision of muscle sampling. Either the left or right quadriceps muscle was used in a random manner. The primates were maintained in a temperature and humidity regulated room and allowed free access to water and to expanded complete primate diet with additional daily fruit supplement and were examined daily to monitor for any

abnormal clinical signs. The clinical observation procedure included the description of animal behaviour (e.g., normal movements, absence of unusual posture or lethargy), condition of the fur, absence of visible wounds, normal respiratory rhythm, aspect of the stools, monitoring for changes in food consumption or signs of pain or discomfort (e.g., favouring of individual limb). Four monkeys from each of the two groups were sacrificed 85, 169 or 366 days (3, 6 or12 months) after the single intramuscular injection. As part of the full necropsy procedure, a macroscopic examination of the injected site was performed to detect any sign of local intolerance.

2.3. Immune response measurement

The humoral immune response to Diphtheria- and Tetanus- antigen was evaluated before immunisation and at necropsy (i.e., 3, 6 or 12 months after immunisation) as a quality control of vaccine administration and to confirm the immunogenicity of the vaccine in the selected species.

2.3.1. Titration of sera for neutralizing antibodies to Diphtheria toxin

Diphtheria toxin neutralizing antibody titres were determined by an in vitro neutralisation assay. Dilutions of sera were incubated with the toxin and the amount of neutralising antibody was estimated using the specific linkage to the toxin and the subsequent inhibition of its cytopathic effect (CPE) on Vero cells. The serum titre corresponded to the highest reciprocal dilution that induced total neutralization of the toxin, as demonstrated by the absence of CPE. The WHO equine international standard was run in parallel. This confirmed the validity of the test and allowed the results to be expressed in International Unit (IU). The seropositivity threshold was defined as an antibody titre equal to or greater than 0.01 IU/ml as used for humans.

2.3.2. ELISA method for measurement of Tetanus IgG antibodies

ELISA for determination of Tetanus toxoid IgG was based on the binding of antibodies to Tetanus toxoid coated polystyrene immunoassay plates, using a series of two-fold dilutions of serum samples. After incubation, they were reacted with peroxidase-conjugated mouse anti-human IgG. The binding was then visualized with *O*-phenylenediamin dihydrochloride as substrate for the peroxidase. The titres of the sera were determined with reference to the WHO International Standard, which was run in parallel. It was therefore possible to express the results in IU. The seropositivity threshold was defined as an antibody titre equal to or greater than 0.01 IU/ml as used in humans.

2.4. Tissue collection and preparation for histopathological examination

Samples taken from Ilio-femoral lymph-nodes, quadriceps muscles and spleens were processed and examined. The muscle fragments obtained from the injected side were oriented along the fibre axis and 3 fragments per muscle were collected (1) in the injection area, (2) in the proximal region, (3) in the distal region. One fragment from the contralateral quadriceps was also sampled as a control. Each fragment was then divided into two pieces, one for histopathology and the second for the microanalysis of metals and mineral ions. Tissue fragments were cryofixed in isopentane chilled with liquid nitrogen. The samples for histological examination were mounted in OCT and two sections were cut on a cryostat, sections were stained with haematoxylin and eosin and submitted for examination. Following these initial examinations, the frozen samples of the muscle from all sites and from all phases of the study were immersed in 10% formalin and allowed to thaw. The muscles fixed in this manner were then processed into paraffin wax. Four to five micron sections were cut and stained with haematoxylin and eosin. Sections were taken at five levels each separated by some 20 μm to ensure that the site of injection was not accidentally missed. In addition to this procedure, samples of the injection site of animals from the 1-year time point of the study which showed no major changes in either the frozen sections or in the five paraffin sections were submitted for further sectioning at 20-μs intervals; this yielded an additional 16–20 sections per animal which were also examined.

2.5. Nuclear microprobe analysis of muscle tissues

2.5.1. Sample preparation

The corresponding samples were stored in cryotubes, transported under dry ice and stored at low temperature (−80 °C) until assay. Tissue sections (thickness ∼20 μm) were obtained using a cryo-microtome equipped with a stainless steel blade. The specimens were cut at low temperature (−30 °C), mounted on fresh formvar films (thickness 0.4 μm) and kept in the cryostat for 6 h until completely freeze-dried. Serial sections were performed under control of light microscopy in order to delineate regions with lesions. Frozen sections for microanalysis were selected during the sectioning procedure on the basis of light microscopy. If reactive zones were observed, the microanalysis was focused on such areas. Where no lesions were observed, mean concentrations were derived from zones about one square millimetre in area.

2.5.2. Nuclear microprobe analysis of tissue sections

The microanalysis of tissue sections was performed using the CENBG nuclear microprobe [21]. A 2.5 MeV proton beam focus down to a 3 μm × 3 μm spot was scanned over 1 mm × 1 mm areas in order to determine the content and the distribution of aluminium and phosphorus together with other types of metal and mineral (Na, Mg, S, Cl, K, Ca, Fe). Particle induced X-ray emission (PIXE) and Rutherford backscattering spectrometry (RBS) analyses were carried out simultaneously to determine both the elemental content and the organic mass of tissue sections. Using this method, metal concentrations in tissue could be accurately calculated according to the

procedure already described [22]. Concentrations of Al and other elements were expressed in terms of dry tissue weight [21].

2.5.3. Data reduction

after analysis, the data were treated as follows: elemental maps were obtained to identify areas where abnormal phosphorus or aluminium levels appeared. Where histologically abnormal structures were found, different zones of interest were delimited and local spectra were extracted. After data reduction, mean Al and P concentrations could be calculated in the selected structures. Two types of analysis were performed. For sections with normal histology, the mean concentrations were calculated over the whole scanned area. Where lesions appeared in the elemental maps, the mean concentrations were calculated in both the reactive area (usually with high Al content) and the neighbouring region (Fig. 1).

3. Results

3.1. Immunogenicity

All the monkeys were negative for both Diphtheria and Tetanus antibodies before vaccination and then seroconverted after vaccination (geometric means in UI/ml, $n = 10–12$ i.e., 0.003 versus 0.114 and 0.006 versus 0.488 for Diphtheria and Tetanus antibody titers, respectively before and after vaccination with ALOOH adjuvanted vaccine; 0.003 versus 0.246 and 0.005 versus 0.707 for Diphtheria and Tetanus antibody titers, respectively before and after vaccination with ALPO4 adjuvanted vaccine). Due to the limited number of monkeys, no statistical analyses were performed between the ALOOH and ALPO4 adjuvanted vaccine treated groups.

3.2. Results of the histopathological examination

Both cryostat and subsequent paraffin sections of each muscle were examined. The paraffin sections obtained from the thawed material had a well-observed morphology. The changes seen in the cryostat sections were in general also present in the paraffin sections. Lesions seen at each of the five levels were similar in all cases with only minor variations in the distribution of certain elements of the lesion.

The true orientation of the samples was not always easy to discern. In general, the lesion was on the cut edge and usually in the region of 5 mm from the identified top of the sample. With both adjuvanted vaccines, the injected suspension was taken up by macrophages, which were concentrated at the centre of the injection site, but showed some extension along the fascia between the adjacent muscle bundles.

In all cases in the initial sacrifice on day 85 (3 months), macrophage aggregation was graded as moderate to marked (Fig. 2A and B). This reaction was accompanied by a lymphoid infiltration. The macrophages having taken up the material showed various degrees of degeneration in the sites with both products. Only in one of the monkeys, given AlOOH adjuvanted vaccine, had this progressed into a cyst-like structure lined by macrophages and fibrocytes. The size of the inflammatory lesion was greater in those monkeys given the ALOOH adjuvanted vaccine.

No significant lesions were observed in the contralateral muscle or in the proximal or distal samples of the injected quadriceps.

Six months after the vaccine injection, on day 169, the four monkeys given the AlPO4 adjuvanted vaccine all showed a minimal residual lymphoid infiltration and/or focal fibrosis but no macrophages were present. These minor lesions are considered to confirm that the injection site had been correctly sampled. In the sites injected with the AlOOH adjuvanted vaccine, one monkey showed no lesions, however, 3/4 monkeys showed appreciable lesions composed mainly of macrophages (Fig. 2C). In one, there was an extensive cyst-like structure lined by macrophages and containing degenerate macrophages (Fig. 2D). This suggested that the macrophages, which had taken up the injected suspension, had degenerated releasing the material, which was then taken up by new macrophages in the cyst lining leading to persistence at the site of injection.

One year following the injection, no macrophage aggregations were seen in monkeys injected with the AlPO4 adjuvanted vaccine. Minor and potentially incidental lymphocytic infiltrations were noted.

In the monkeys given the AlOOH adjuvanted vaccine, two of the four monkeys still had moderate macrophage aggregations with associated minor lymphocytic infiltrations (Fig. 2E and F).

Extensive further sections (covering some 500 μm) on negative samples did not reveal any major lesions but only small foci of lymphocytes, which were considered to be incidental and not associated with the injection (Table 1).

No abnormal findings were observed for the spleens and lymph nodes from all animals.

3.3. Nuclear microprobe analysis of tissue sections

3.3.1. Analyses of injection sites from the injected quadriceps (Table 2)

Analyses of injection sites from the injected quadriceps (Table 2): 3 months after injection, lesions were observed in all samples taken from injection sites of quadriceps mus-

Table 1
Mean concentrations of aluminium and phosphorus in the zones of interest delimited in Fig. 1

Zone of interest	Aluminium (μg/g)	Phosphorus (μg/g)	Zones
Zone a	2140	12330	Reactive
Zone b	4010	20530	Reactive
Zone c	120	6830	Neighbouring
Zone d	190	9350	Neighbouring
Zone e	100	9770	Neighbouring

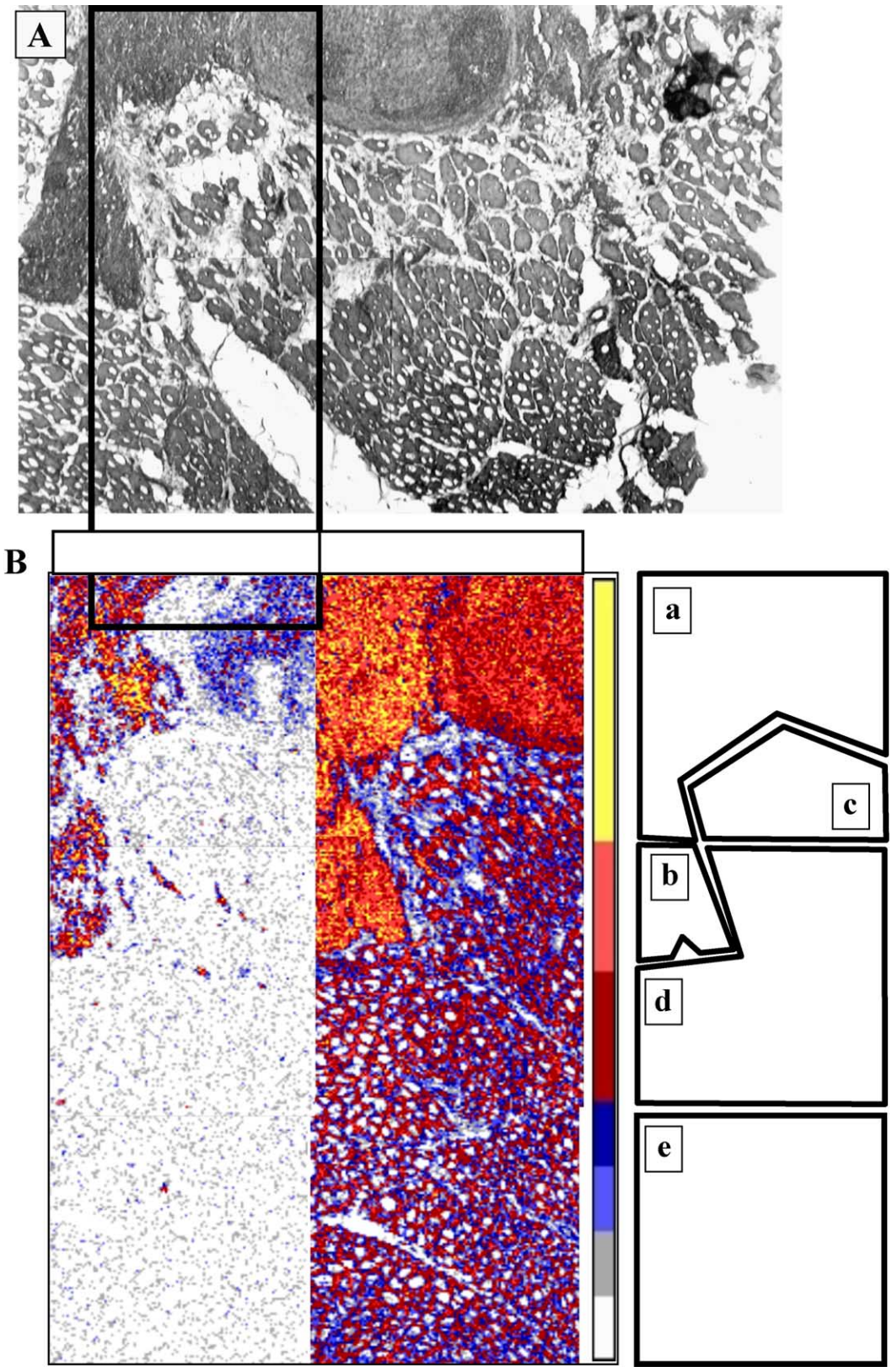

Fig. 1. Nuclear microprobe analysis of tissue section. (A) Identification of a potential lesion (i.e., upper part of this heamatoxylin and eosin stained slide prepared from a muscle taken 3 months after injection of the $AlPO_4$ adjuvanted vaccine). (B) Distributions of aluminium and phosphorus after PIXE microanalysis of the area delimited by the frame indicated (A). The concentration increases from white to yellow on the colour scale (size of the analysed area 3 mm × 1 mm). The zones of interest being considered for the calculation of concentrations (see Table 1) are presented in the right part of the figure.

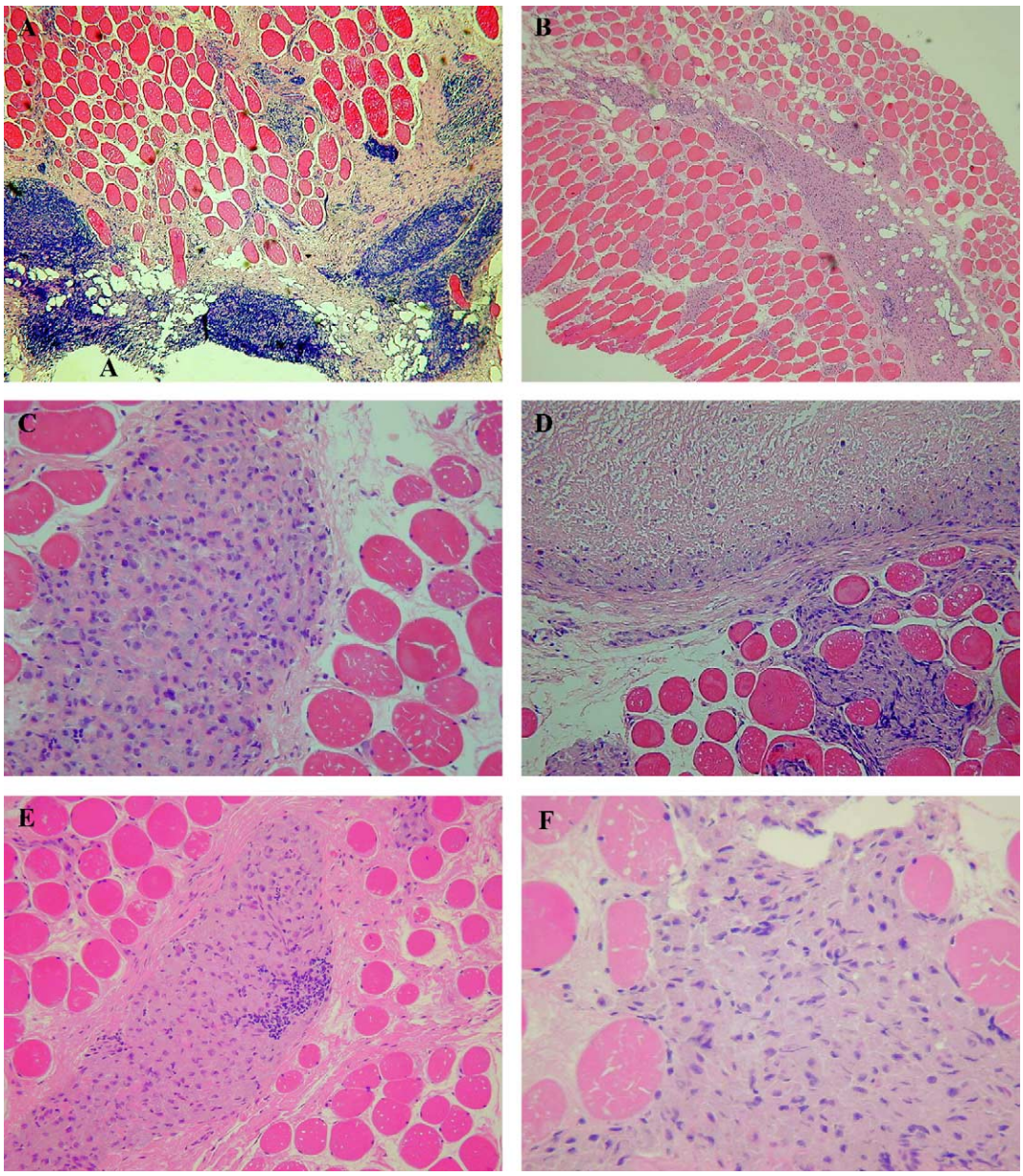

Fig. 2. Histopathological examination of the injection site of aluminium adjuvanted vaccines (haematoxylin and eosin stain). (A) 3 months after AlPO4 adjuvanted vaccine injection (low power), (B) 3 months after AlOOH adjuvanted vaccine injection (low power), (C and D) 6 months after AlOOH adjuvanted vaccine injection (high and medium power), (E and F) 12 months after AlOOH adjuvanted vaccine injection (medium and high power).

cles and selected for nuclear microprobe analysis as reported previously for the samples used for the histopathological examination. High aluminium content was found for both AlPO4 and AlOOH adjuvanted vaccine treated animals. Aluminium concentration in the reactive zones of animals treated with AlOOH was four times higher than in those treated with AlPO4. In neighbouring zones, the aluminium concentration decreased down to a few hundred micrograms per gram within 0.5–2 mm from the border of reactive zones. Phosphorus was found to be highly correlated with aluminium in reactive zones (see elemental maps, Fig. 1). The mean concentration in these areas was higher after immunisation with AlPO4 than with AlOOH adjuvanted vaccines (14370 ± 2540 μg/g versus 11350 ± 2930 μg/g) presumably due to the phosphorus in the adjuvant. Both values were significantly higher than in neighbouring regions which were equivalent to that of tissue from contralateral muscles.

No lesions were observed in AlPO4 adjuvanted vaccine injection sites six months after injection (four animals) confirming the findings in the other fragments taken from the same site. For these four animals, in 21 out of 33 sections, the aluminium concentration was above the detection limit of the method (25 μg/g) and under the limit in the remaining 12

Table 2
Mean concentrations of aluminium and phosphorus in the injection site of quadriceps for eight animals sacrificed 3 months after injection and for eight animals sacrificed after 6 months

Adjuvant	Analysed zones	Al (μg/g)	P (μg/g)	No. of animals	No. of analyses
AlPO 43 months	Reactive	2860 ± 10570	14370 ± 2540	4	18
	Neighbouring	410 ± 445	10120 ± 1960	4	15
AlOOH 3 months	Reactive	14280 ± 7130	11350 ± 2930	4	25
	Neighbouring	680 ± 595	9460 ± 1170	4	18
AlPO 46 months[a]	No apparent lesions	147 ± 90	7290 ± 3310	4	21
AlOOH 6 months	Reactive	11000 ± 8430	10090 ± 2900	2	7
	Neighbouring	167 ± 87	8730 ± 960	4	14[b]

The results are compared for both AlOOH and AlPO4 adjuvanted vaccines. The mean values are given in μg/g of dry weight (±S.D.) after analysis of the reactive zone and of neighbouring regions (distance from the lesion ranging from 0.5 to 2 mm). Six months after injection, the concentration of aluminium in several sections was found to be under the detection limit for the technique: see notes (a) and (b).

[a] Plus 12 analysed sections for which the Al concentration was under the detection limit (approximately 25 μg/g), and not taken into account in the mean Al concentration.

[b] Plus 4 analysed sections for which the Al concentration was under the detection limit (approximately 25 μg/g) for the 2 animals without reactive region and not taken into account in the mean Al concentration.

sections. The level of phosphorus concentration was slightly lower than in the contralateral muscle.

In two animals treated with AlOOH adjuvanted vaccines, residual lesions were found in injection sites. The aluminium concentration was still very high in these reactive zones. In neighbouring regions, the aluminium level was lower than that observed in similar areas at 3 months. The concentration in these regions was at the same level as in sections which had no apparent lesions obtained from the two remaining animals. Finally, a mean aluminium concentration was calculated taking in regions in the neighbourhood of lesions together with sections showing no apparent lesion. No significant difference was found in the aluminium level when compared with tissues of animals treated with AlPO4 adjuvanted vaccines at six months. Six months after injection, the phosphorus concentration in reactive zones of AlOOH treated muscles decreased down to a level comparable to values obtained in contralateral tissues.

At 12 months, the aluminium level was under the detection limit in all sections for both AlPO4 and AlOOH.

3.3.2. Analyses of proximal and distal samples from injected quadriceps

Analyses of proximal and distal samples from injected quadriceps: for proximal and distal samples of injected quadriceps, since no apparent lesions were revealed histologically in frozen sections whatever the sacrifice time 3, 6 or 12 months, areas were chosen for analysis randomly. The analyses were carried out as follows: (i) 8 analyses on proximal samples and 9 analyses on distal samples 3 months after injection, (ii) 22 analyses on proximal samples and 25 analyses on distal samples 6 months after injection. No samples were analysed for animals sacrificed 12 months after injection. For most sections, the aluminium level was found to be under the detection limit. In 5 analyses (3 distal AlPO4 and 2 distal AlOOH sections 6 months after injection) a few small aluminium-containing granules were observed spread throughout the tissue. The mean phosphorus concentration did not differ significantly from that in contralateral tissues: 9670 ± 500 μg/g (3 months after injection) and 10,690 ± 700 μg/g (6 months after injection) in AlPO4 samples; 9420 ± 400 μg/g (3 months after injection) and 10,050 ± 650 μg/g (6 months after injection) in AlOOH samples.

3.3.3. Analyses of contralateral quadriceps

Analyses of contralateral quadriceps: no apparent lesions were observed in frozen histological sections obtained from contralateral quadriceps muscles 3 months after injection. A dozen sections were analysed. The aluminium concentration was found to be under the detection limit in all tissue sections (<25 μg/g) whereas the mean phosphorus concentration was 9500 ± 450 μg/g. Contralateral quadriceps at 6 and 12 months were not analysed.

4. Discussion

Despite the extensive use of aluminium salts as adjuvants, little is known about their pharmacokinetics and the majority of the data comes from aluminium exposure through environmental sources. Biokinetics studies showed that free aluminium bound mainly to transferrin and citrate [23]. In an in vitro study [24], aluminium adsorbed to a mock antigen was rapidly separated by free interstitial proteins, the opposite effect was not observed (i.e., separation of interstitial proteins bound to aluminium by an antigen). Ultimately, elimination data [19,23] indicated that aluminium is cleared with a rapid initial release followed by a subsequent long-term process. Bone is the main tissue responsible for long-term storage.

Our protocol did not evaluate all body compartments neither did it include an in situ measurement of the total quantity of Aluminium injected. The measurement performed was a

quantitative evaluation of aluminium distribution in muscle sections. However, the observed increase in aluminium concentration associated with the histopathological changes in the muscle support the hypothesis that aluminium from the vaccines remains at the site for at least 6 months. This increase is likely to be directly related to the injected vaccine. The hypothesis that this additional aluminium could come from a source other than the vaccine is highly improbable as demonstrated by other authors from ^{26}Al in the rat (Authier, manuscript in preparation). This persistence at the injection site would seem to be longer than expected considering the fact that the muscle is not generally considered to be a retention organ whereas bone is. The lack of detection of an increase in aluminium concentration 12 months after injection of both adjuvanted vaccines cannot be definitively considered to demonstrate total clearance of the adjuvant from the muscle. A focal area of increase in aluminium level could potentially not have been sampled.

The second essential finding of this monkey study was the aspect and the persistence of the histopathological lesion observed at the injection site. This lesion characterized by macrophage aggregates between the muscular fibres with extension along the fascia and associated with lymphoid infiltration is similar to the lesion observed in some patients and termed macrophagic myofasciitis [13,14]. These observations would lead to the conclusion that this type of lesion is a usual reaction following the injection of an aluminium adjuvanted vaccine by the intramuscular route.

Interestingly neither behavioural changes nor any signs of muscular weakness were observed in the vaccinated monkeys at any time.

Another characteristic of this lesion is its localisation around the injection site; no changes were observed neither in the distal or proximal muscle fragments sampled 20 mm from the injection site nor in lymph nodes or spleen. One can conclude it would be difficult to observe this lesion if either the injection site were not clearly identified or if muscle fragments examined were not taken close to the injection site. No changes were observed in the contralateral muscle. The focal pattern of this lesion does not support the hypothesis of a more widespread muscular disease.

Macrophage aggregates were still observed in two out of four monkeys one year after injection of the AlOOH adjuvanted vaccine. This persistence could demonstrate depot formation to be one of the mechanisms of action for the adjuvant effect of aluminium as has been proposed. However, we did not develop a method, which would also verify whether the antigen is still present in these lesions.

Based on the results of this study, we conclude that macrophagic myofasciitis lesions can occur in normal healthy animals and can be associated with both AlPO4 and ALOOH adjuvanted vaccines. However, lesions persist longer with AlOOH. The hypothesis that the vaccine material is taken up by macrophages, which are then replaced by other macrophages, seems plausible considering the persistence of the macrophage aggregates.

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EXHIBIT 363

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INORGANIC COMPOUNDS

Aluminium in plasma and tissues after intramuscular injection of adjuvanted human vaccines in rats

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Abstract
Aluminium (Al) toxicokinetics after intramuscular (IM) injection of Al-adjuvanted vaccines is unknown. Since animal data are required for modeling and extrapolation, a rat study was conducted measuring Al in plasma and tissues after IM injection of either plain Al-hydroxide (pAH) or Al-phosphate (pAP) adjuvant (Al dose 1.25 mg), single human doses of three Al-adjuvanted vaccines (V1, V2, and V3; Al doses 0.5–0.82 mg), or vehicle (saline). A significant increase in Al plasma levels compared to controls was observed after pAP ($AUC_{(0–80\ d)}$, mean ± SD: 2424 ± 496 vs. 1744 ± 508 µg/L*d). Percentage of Al dose released from injected muscle until day 80 was higher after pAP (66.9%) and AP-adjuvanted V3 (85.5%) than after pAH and AH-adjuvanted V1 (0 and 22.3%, resp.). Estimated absolute Al release was highest for pAP (836.8 µg per rat). Al concentration in humerus bone was increased in all groups, again strongest in the pAP group [3.35 ± 0.39 vs. 0.05 ± 0.06 µg/g wet weight (ww)]. Extrapolated amounts in whole skeleton corresponded to 5–12% of the released Al dose. Very low brain Al concentrations were observed in all groups (adjuvant group means 0.14–0.29 µg/g ww; control 0.13 ± 0.04 µg/g ww). The results demonstrate systemically available Al from marketed vaccines in rats being mainly detectable in bone. Al release appears to be faster from AP- than AH-adjuvants. Dose scaling to human adults suggests that increase of Al in plasma and tissues after single vaccinations will be indistinguishable from baseline levels.

Keywords Aluminium · Adjuvants · Systemic availability · Rats · Intramuscular · Vaccine

Introduction

Aluminium (Al) compounds have been widely used for decades as adjuvants in vaccines. They mainly consist of complex morphologies of crystalline Al-oxyhydroxide or amorphous Al hydroxyphosphate (Hem and HogenEsch 2007) referred to below for the ease of reading as Al-hydroxide ("AH") and Al-phosphate ("AP"). The poorly soluble adsorbents are commercially available as wet gel suspensions (e.g., Alhydrogel® or Adju-Phos®) or are produced by vaccine manufacturers themselves. Many human vaccines are adsorbed on AH or AP, e.g., the toxoid vaccines against diphtheria and tetanus, acellular pertussis, hepatitis B, pneumococcal and meningococcal vaccines, potentiating the immune response to the poorly immunogenic antigens, thereby enabling successful vaccination. Al content in human vaccines is limited to 1.25 mg per dose by WHO (WHO 2016) and European Pharmacopeia (Ph. Eur. 2018), and is labeled in the product information.

Although to date there is no scientific evidence for a causal relationship between Al containing vaccinations and acute or chronic neurological impairment or diseases (Immunization Safety Review 2001, 2004; WHO 2012), there is still concern about the potential toxicity on the central nervous system or bone deriving from vaccine exposure.

Remaining uncertainty could at best be erased by better knowledge of toxicokinetics after intramuscular (IM) injection of Al-adjuvanted vaccines. While Al bioavailability

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after parenteral administration is supposed to be 100%, the rate of absorption and thus potential Al increase in plasma and tissues over time in man is unknown. A few investigations in rabbits and monkeys suggest that AP has a higher rate of bioavailability than AH (Flarend et al. 1997; Verdier et al. 2005).

A physiology-based toxicokinetic (PBTK) model is urgently needed for extrapolation of animal data to humans (Krewski et al. 2007). However, relevant animal data on Al absorption and distribution after administration of Al-adjuvanted products to inform such a model are lacking (Weisser et al. 2017; Masson et al. 2018).

We therefore aimed at collecting data on Al bioavailability from adjuvants in vivo by injecting a full human dose of unmodified marketed vaccine products IM into rats. Vaccines should represent both adjuvant types at the highest available Al content per dose. Since most studies investigating Al toxicokinetics from soluble species have been conducted in rats (Weisser et al. 2017, 2019), also with regard to model building this species was considered most appropriate. We monitored Al concentrations in plasma, at the injection site, in bone, and in whole brain hemisphere up to 80 days post-injection.

Materials and methods

Animals

In vivo studies in male Wistar rats (approx. 2 months; body weight 350 g ± 65 g, Charles River Labs, Sulzfeld) were conducted by preclinics GmbH (Potsdam, Germany).

Rats were randomly assigned to treatment groups (no allocation parameter) and were allowed free access to tap water and standard diet [R/M-H, extruded (V1536), Ssniff, Soest, Germany]. The animals were kept under 12 h/12 h light–dark cycle conditions. After 19 days of acclimatization following arrival, animals were anesthetized with 5 vol % isoflurane (IsoFlo 100%; Ecuphar GmbH, Greifswald) and blood was collected from the lateral tail vein to obtain the blank value. Thereafter, treatment preparation or vehicle solution was administered according to the schedule described under treatment.

Rats were housed and handled according to guidelines from the Federation of Laboratory Animal Science Associations (FELASA). The animal study was performed in compliance with the German animal protection law and was registered at the Landesamt für Umwelt, Gesundheit und Verbraucherschutz Brandenburg.

Treatment preparations

Vaccine products (V1, V2, V3) were purchased at a local pharmacy. All three products are marketed in the EU, adjuvanted with either AH (V1), AP (V3) or both AH and AP (V2). A single human dose (0.5 mL) of each vaccine was applied containing 0.5–0.82 mg Al (Table 1). If applicable, fresh preparation was done as indicated in the product information.

Plain adjuvant suspensions (pAH and pAP) were prepared from commercial gels (Alhydrogel® 2% and Adju-Phos®; Brenntag Biosector A/S, Frederikssund, Denmark) by dilution with sterile saline to achieve an Al concentration of 1.25 mg per 0.5 mL. Suspensions were freshly prepared within 24 h and thoroughly vortexed before administration.

Treatment

Each rat received 0.5 mL of either a self-prepared plain adjuvant suspension (pAH or pAP) or a vaccine (V1, V2, or V3; Table 1). A control group receiving 0.5 mL sterile saline (vehicle) was run to monitor the underlying plasma Al steady-state concentration over time ("baseline") resulting from dietary Al intake. Al contamination of the saline vehicle solution was controlled and found negligibly small (≤ 2.5 ng in 0.5 mL). In all rats the injection volume of 500 µL was administered intramuscularly via six injection sites (100 µL each into both M. quadriceps and M. gastrocnemius of the hind limbs and 50 µL each into both M. triceps of the front limbs).

Table 1 Overview of study groups and treatment

Group ID	Animals per group (N)	Treatment preparation	Route of administration	Injection volume[a]	Al dose (mg per animal)	Al dose (mg/kg)
pAH	7	Alhydrogel®-suspension	IM	0.5 mL	1.25	3.6
pAP	6	Adju-Phos®-suspension	IM	0.5 mL	1.25	3.6
V1	7	AH-adjuvanted vaccine	IM	0.5 mL	0.6	1.7
V2	7	AH/AP-adjuvanted vaccine	IM	0.5 mL	0.82	2.3
V3	6	AP-adjuvanted vaccine	IM	0.5 mL	0.5	1.4
Vehicle	6	Saline	IM	0.5 mL	–	–

[a]Administered via 6 sites

Sample collection

Blood samples (approx. 300 µL) were collected from the lateral tail vein at pre-dose, and at day 1, 5, 10, 15, 20, 30, 45, 60, and 80 post-dose using K3-EDTA Multivette 600 collection tubes (Sarstedt, Nümbrecht) connected to a 23G cannula. Blood was centrifuged at 4 °C for 10 min at 3220×*g*. Plasma was pipetted into 1.5 mL microtubes and stored at − 20 °C. In all rats, at time of euthanasia [80 days p.i. (post-injection)] the right hemisphere of the brain, whole muscle *M. triceps* and whole humerus bone of the right front leg were dissected, transferred into 5 mL tubes, weighed, and stored at − 70 °C.

Bioanalytical method

Measures taken for contamination control and the bioanalytical method used for determination of total Al concentration in plasma and tissues (AAS) were as described in detail in a previous publication (Weisser et al. 2019). The whole pre-analytical and analytical process was designed and controlled for minimizing Al contamination. All determinations in the analytical laboratory were conducted in blinded manner. Al concentration in bone was determined as µg/g wet weight (ww), in muscle and brain samples as both µg/g ww and µg/g dry weight (dw).

Data analysis

Individual area under the curve (AUC) of Al in plasma from zero to day 80 ($AUC_{(0–80\ d)}$) was calculated by the linear trapezoidal rule (MS excel).

Individual Al concentration (µg/g) measured in muscle samples were multiplied by the wet weight of the muscle sample (g) to give the absolute Al amount in whole *M. triceps* (µg). Al dose "remaining" (%) was calculated as the ratio between Al amount in whole *M. triceps* (subtracted by vehicle group mean) and Al dose injected into *M. triceps*. Al dose "released" (%) was calculated as 100 - Al dose "remaining" (%). Under the assumption of equal absorption behavior in all six injection site muscles total absolute Al "release" in µg per rat was estimated as percentage Al dose "released" in *M. triceps*/100 × total Al dose injected on day 0. Individual negative ratios were not set to zero.

Statistical analysis

If not otherwise indicated, data are presented as means ± standard deviation (SD). Statistical tests were calculated for a two-sided significance level $\alpha = 0.05$, adjusted for multiple comparisons where necessary.

Two plasma and one muscle sample showing implausible high Al concentrations were eliminated as outliers (confirmed by Dixon's outlier test).

To investigate stability of Al plasma concentration in the vehicle group over time, a linear trend curve was fitted to the data from day 0 up to day 80 by means of a linear model for repeated measures (animal) with fixed factor day.

Testing for a significant difference of Al plasma exposure after treatment compared to vehicle group was done by comparison of total $AUC_{(0–80\ d)}$ (Wilcoxon–Mann–Whitney test, two-sided). Percent remaining Al concentration at injection site was tested for a significant difference from 100% by the Wilcoxon signed rank test.

Al concentration in bone or brain samples was compared between groups using a linear model (ANOVA) with fixed factor "treatment" based on logarithmized values. The statistical analysis was performed with SAS®/STAT software, version 9.4, SAS System for Windows, and software *R*.

Linear regression and correlation (Pearson r) analysis were done by GraphPad Prism® (Version 7.04) software.

Results

All rats tolerated treatments well and did not show any sign of toxicity throughout the study.

Al in plasma

Mean total Al plasma concentrations over time up to day 80 and calculated plasma $AUC_{(0–80\ d)}$ for all treatment groups are shown in Fig. 1 and Table 2.

Mean pre-treatment levels of Al concentration in plasma were similar in all groups (overall mean 12.4 ± 7.8 µg/L). The mean concentration of the vehicle control group over 80 days was 19.8 µg/L (95% CI 14.4–25.3; CV 82%; geometric mean: 14.3 µg/L; 95% CI 10.8–19.0) showing a slightly positive slope of the time course (0.177, p = 0.0298).

Al plasma time courses after treatment did not exhibit profiles distinctive from that of the vehicle group, except the pAP curve showing an apparent peak on day 10 with a maximum Al difference to baseline of about 30 µg/L. Total Al plasma exposure in terms of $AUC_{(0–80\ d)}$ was significantly enhanced in the pAP, but not in other groups, compared to vehicle with a mean absolute difference of 681 µg/L*d.

Al in tissues

Injection site muscle

None of the IM-treated animals showed palpable indurations at the injection sites throughout the study.

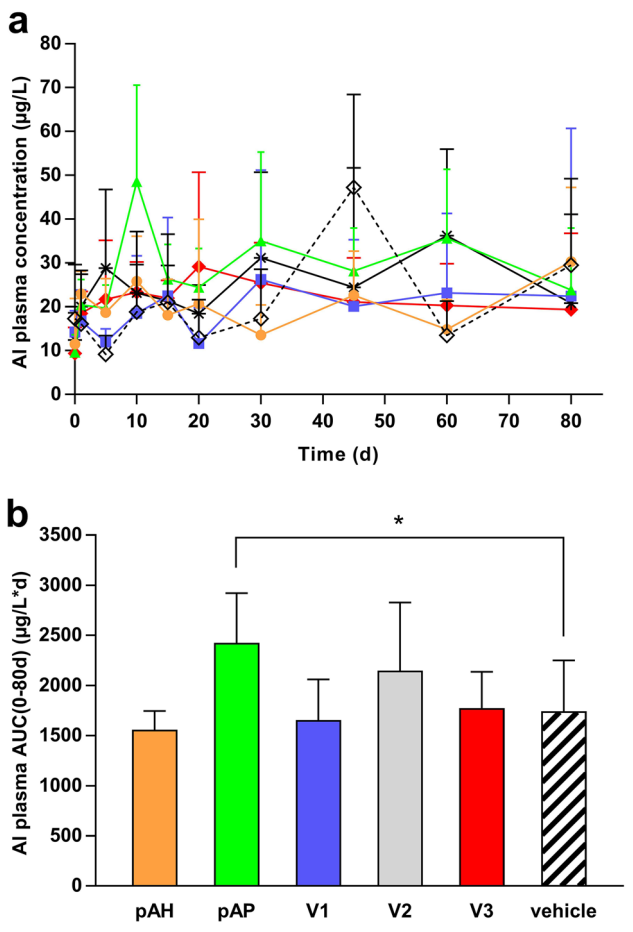

Fig. 1 Mean (+SD) Al plasma concentration–time course (**a**) and Al plasma $AUC_{(0–80\ d)}$ (**b**) in rats after IM administration of pAH (filled circles), pAP (filled triangles), V1 (filled squares), V2 (asterisks), V3 (filled diamonds), or vehicle (open diamonds; dotted line). *$p < 0.05$ (Wilcoxon–Mann–Whitney test on difference to vehicle)

Results of total Al amounts measured in one injection site muscle (*M. triceps*) on day 80 and calculated fractions of Al dose "remaining" and "released" from *M. triceps* compared to the injected dose (1/10 of total Al dose) are shown in Table 2 and Fig. 2.

After treatment with pAH total injected Al amount was completely recovered in *M. triceps* at day 80 (102.1%), whereas mean percentage Al "remaining" in the pAP group was 33.1% only. In contrast to V1 (77.7%), the percentage Al "remaining" was also significantly below 100% in groups V2 and V3 (68.2 and 14.5%, respectively; Fig. 2a).

The highest percentage Al dose "released" from the injection site was found in group V3 (85.5%) followed by pAP (66.9%). Due to the higher Al dose injected, the highest absolute Al amount released from all injection site muscles was estimated for pAP (836.8 µg) followed by 427 mg for V3 (Fig. 2b and Table 2).

Bone

In all treatment groups geometric mean Al bone concentration at day 80 p.i. was significantly higher than in the vehicle controls (all p values < 0.001; Table 2 and Fig. 3a). Variability in the treatment groups was low (CV 11.6–62.4%). Maximum geometric mean Al concentration found was 3.33 µg/g ww (pAP group) which amounts to an absolute difference of 3.28 µg/g ww compared to GM in vehicle controls (0.05 µg/g ww). Absolute GM differences were 2–15 times lower (1.40, 1.23, 0.76, and 0.22 µg/g ww) in V3, V2, pAH, and V1 group, respectively.

Brain

Geometric mean Al concentration in the right brain hemisphere was below 0.3 µg/g ww (1 µg/g dw) in all groups with low inter-individual variability (CV < 36%; Table 2 and Fig. 3b). In three groups (V1, V2, and V3) statistically significant differences to vehicle were observed (Table 2).

Relationship between estimated Al release and plasma/tissue exposure

A positive relationship was found between estimated Al amount released from all injection sites and exposure observed in plasma and bone in all adjuvant treated rats (Fig. 4). For both plasma $AUC_{(0–80\ d)}$ ($y = 0.57x + 1737$; $r = 0.35$; Fig. 4a) and bone Al concentration ($y = 0.0025x + 0.61$; $r = 0.78$; Fig. 4b), a linear increase with total Al release was found.

Discussion

To our knowledge this are the first data demonstrating systemic increase of Al concentrations, particularly in bone, after IM administration of marketed Al-adjuvanted human vaccines in vivo. Though Flarend et al. (1997) investigated short-term plasma and various tissue Al concentrations in two rabbits, they did not evaluate Al levels in bone and used intramuscular (IM) injection of plain self-prepared ^{26}Al-adjuvants (Masson et al. 2018). Their results indicated an increase in Al plasma levels of 1–2 µg/L after a dose of 0.28 mg Al/kg in rabbits. Going beyond, we administered the highest Al adjuvant dose allowed in human vaccines (1.25 mg; WHO 2016; Ph. Eur. 2018) as well as full human doses of marketed human vaccines in rats reaching much higher Al doses in relation to body weight (1.4–3.6 mg/kg). Furthermore, we measured Al in bone being the major storage compartment of Al in both animals and humans (Yokel and McNamara 2001; Priest 2004; Krewski et al. 2007).

Table 2 Al plasma $AUC_{(0–80\ d)}$ and Al amounts measured in injection site muscle, bone and brain on day 80 after injection of plain adjuvants (pAH, pAP), adjuvanted vaccines (V1–V3), or vehicle in rats (mean and standard deviation (SD); coefficient of variance (CV); geometric mean (GM))

Treatment group	Plasma	Injection site muscle				Bone	Brain
	Al plasma $AUC_{(0–80\ d)}$ (µg/L*d)	Al amount injected into *M. triceps* (µg)	Al amount in *M. triceps* on day 80 p.i. (µg)	Al "remaining" in *M. triceps* (% admin. dose)	Extrapolated Al release from all injected muscles (µg per rat)	Al concentration on day 80 p.i. (µg/g ww)	Al concentration on day 80 p.i. (µg/g ww [dw])
pAH ($n=7$)							
Mean	**1593**	**125**	**127.7[b]**	**102.1**	**–**	**0.86**	**0.17 [0.60]**
SD	193		17.4	14.0		0.25	0.06
CV (%)	12.1		13.7			28.8	35.7
GM	1549		126.7			0.81	0.16 [0.56]
p-value	0.77[a]			1.00[a]		<0.001[c]	0.41[c]
pAP ($n=6$)							
Mean	**2424**	**125**	**41.4**	**33.1**	**836.8**	**3.35**	**0.14 [0.50]**
SD	496		14.9	11.9	148.8	0.39	0.05
CV (%)	20.4		35.9			11.6	33.7
GM	2382		38.5			3.33	0.13 [0.48]
p-value	0.02[a]			0.03[a]		<0.001[c]	0.98[c]
V1 ($n=7$)							
Mean	**1654**	**60**	**46.7**	**77.7**	**134.1**	**0.34**	**0.23 [0.81]**
SD	407		27.6	46.1	276.3	0.21	0.06
CV (%)	24.6		59.2			62.4	27.7
GM	1607		28.7			0.27	0.22 [0.78]
p-value	0.73[a]			0.38[a]		<0.001[c]	0.007[c]
V2 ($n=7$)							
Mean	**2147**	**82**	**56.0**	**68.2**	**261.1**	**1.30**	**0.29 [1.02]**
SD	682		7.2	8.8	72.0	0.30	0.09
CV (%)	31.8		12.9			23.3	32.3
GM	1979		55.5			1.28	0.28 [0.99]
p-value	0.18[a]			0.02[a]		<0.001[c]	<0.001[c]
V3 ($n=6$)							
Mean	**1776**	**50**	**7.3**	**14.5**	**427.4**	**1.47**	**0.24 [0.80]**
SD	359		1.8	3.6	18.0	0.19	0.08
CV (%)	20.2		24.5			13.2	32.0
GM	1748		7.2			1.45	0.23 [0.77]
p-value	0.53[a]			0.03[a]		<0.001[c]	0.006[c]
Vehicle ($n=6$)							
Mean	**1744**	–[d]	**0.08**	–	–	**0.05**	**0.13 [0.43]**
SD	508		0.04			0.06	0.04
CV (%)	29.1		47.4			125	31.7
GM	1673		0.08			0.02	0.12 [0.41]

Mean values were kept in bold for better visualization

[a]Wilcoxon test (two-sided) on difference to vehicle group or to 100%

[b]$n=6$ only

[c]Evaluation based on linear model for logarithmized values with fixed factor treatment compared to vehicle group

[d]<0.00025 µg (see "Methods")

The treatment preparations comprised plain suspensions of the two adjuvant types AH and AP which are commonly used in vaccine production (HogenEsch et al. 2018) as well as three authorized vaccine products either solely based on AH (V1) or AP (V3), or both (V2). As these products contain the natural ^{27}Al-isotope, our study was designed to

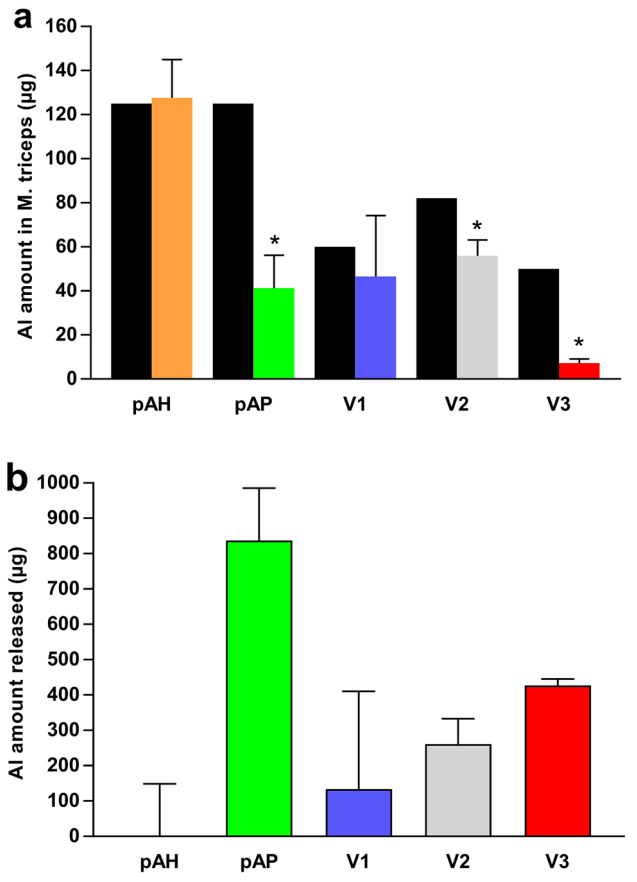

Fig. 2 **a** Mean (+SD) Al amount (difference to vehicle group mean) found in injection site muscle *M. triceps* of rats 80 days after treatment (light/colored bars) compared to Al amount injected into this muscle on day 0 (black bars). *$p < 0.05$ (Wilcoxon signed rank test on difference to 100%). **b** Mean (+SD) extrapolated Al release from all injection site muscles per rat at day 80 p.i

monitor Al "baseline" levels in plasma and tissues resulting from dietary Al intake by use of a control group throughout the whole study period.

After IM application of adjuvanted preparations, only the group treated with plain AP adjuvant showed a significant increase in total Al plasma $AUC_{(0–80\ d)}$ which is a robust quantitative measure of plasma exposure. The mean 80d-baseline plasma level of 19.8 µg/L in our control rats is somewhat higher than that expected in healthy humans (0.5–8 µg/L; Krewski et al. 2007). A lower Al baseline level might have been desirable for the purpose of higher sensitivity to detect AUC differences after treatment. However, we decided against dietary depletion of Al in order not to unbalance the Al equilibrium in the body. The observed slight trend of the baseline towards an increase in slope over time did not have impact on our results, since statistical evaluation in plasma was based on comparison of total AUCs between treatment and control group.

The apparent peak ("Cmax") observed at day 10 after pAP injection is not considered compatible with simple first order absorption kinetics as attempts to estimate an absorption rate constant for pAP by adjusting ka_IM in the recently established model for IM administration of Al citrate (Weisser et al. 2019) was not successful. However, the input process of Al^{3+}ions after injection of insoluble adjuvant particles is probably not characterized by a single kinetic function describing dissolution of the Al complex. Several processes may be involved in parallel [e.g., lymphatic transport of undissolved particles, Al release from immune cells after phagocytosis (He et al. 2015)] causing a substantial delay in the absorption process.

In line with its increase of plasma AUC the pAP group also showed the highest increase of Al concentration in bone (3.28 µg/g ww). However, in contrast to plasma, bone results also indicated systemic availability of Al, though at least twofold less, for all other (including

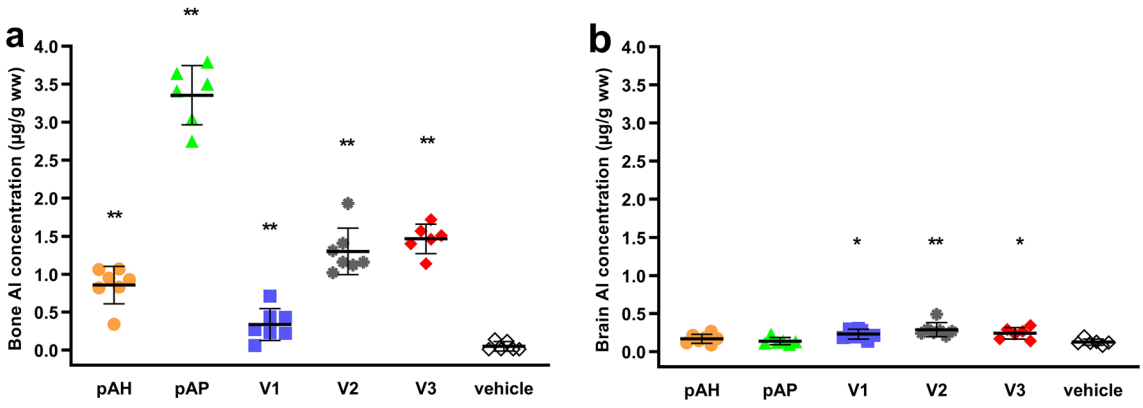

Fig. 3 Al concentration in bone (**a**) and brain (**b**) at day 80 after IM injection of plain adjuvants (pAH, pAP), adjuvanted vaccines (V1–V3), or vehicle in rats. Individual and mean (±SD) levels are depicted. *$p < 0.05$, **$p < 0.001$ (ANOVA compared to vehicle)

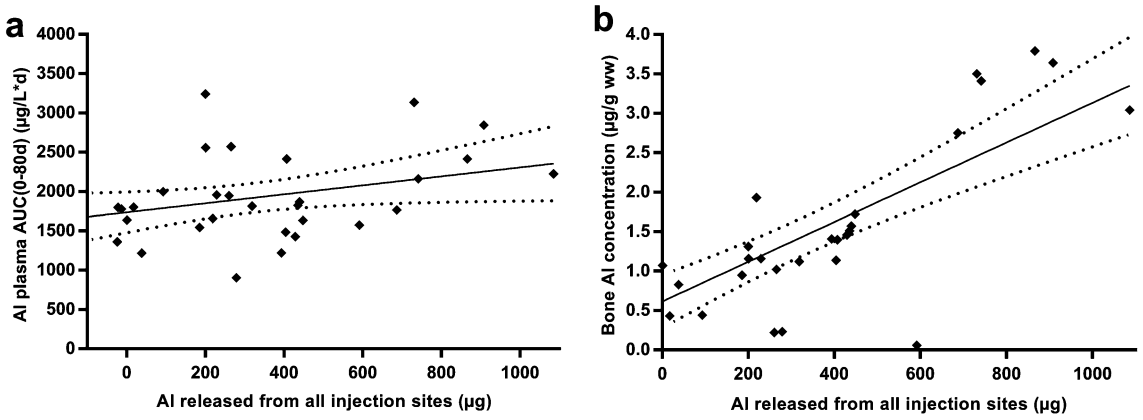

Fig. 4 Relationship between Al amount released from all injection sites and (**a**) Al plasma $AUC_{(0–80\ d)}$ or (**b**) bone Al concentration on day 80 after injection in individual rats (solid line: linear regression curve; dotted lines: 95% confidence limits)

AH-based) formulations. Bone Al levels in the vehicle group (0.05 ± 0.06 µg/g) were extremely low compared to the reference value of 0.53 µg/g ww for healthy rats (mean for all ages; Hirayama et al. 2011). The estimate for the *y*-intercept of the linear relationship found between Al release and bone Al concentration (0.61 µg/g) suggests a higher "true" control level more in line with the reference value.

A more visible increase in bone exposure rather than plasma is not surprising: fast renal Al plasma clearance prevents a sharp rise of plasma levels above a relatively high baseline level, whereas elimination of Al from bone is very slow, thus, Al amounts reaching bone build a long-term deposit which facilitates detection (Yokel and McNamara 2001; Priest 2004; Krewski et al. 2007).

The findings in plasma and bone were confirmed by the injection site release results as an indirect measure of bioavailability up to day 80. A high Al release was noticed for plain AP (66.9%) and AP-adjuvanted V3 (85.5%) in contrast to very small dose fractions of the Alhydrogel®-adjuvanted preparations pAH and V1 (0 and 22.3%, resp.). In accordance with its mixed composition V2 showed a degree of release between both extremes (31.8%). Thus, we observed a remarkable difference in the degree of Al release up to day 80 between AP and AH after injection of plain adjuvants as well as vaccines containing the respective adjuvant type. Crude linear extrapolation from 100% on day 0 through the mean dose fraction of V1 remaining at the injection site on day 80 (77.7%) predicts that complete absorption of Al from AH-adjuvanted vaccines will take at least 350 days (1 year). In contrast, linear extrapolation through the remaining dose fraction for V3 (14.5%) suggests that Al from AP-adjuvanted vaccines might be completed much earlier after ca. 120 days.

Our results are in line with injection site muscle measurements after vaccination in macaques by Verdier et al. (2005) who still observed substantial Al concentration in *M. quadriceps* after injection of the AH-adjuvanted vaccine at 6 months p.i., in contrast to low but significant Al concentrations above control at 3 months (90 d) but no longer at 6 months (180 d) after injection of an AP-adjuvanted vaccine. In contrast to Verdier et al., we collected the whole injected muscle being able to quantify the percentage of injected dose. Our quantitative differences suggest a 3- to 4-fold higher rate of systemic availability for AP than AH. The results are fully in line with the threefold Al plasma $AUC_{(0–28\ d)}$ found after self-prepared plain AP compared to AH in rabbits (Flarend et al. 1997). We could demonstrate that this difference also applies to marketed adjuvanted vaccines.

The disparity is most probably attributed to well-known physicochemical differences between AP and AH, mainly the degree of crystallinity, chemical composition and surface charge: AH consists of crystalline Al-oxyhydroxide (AlOOH), whereas AP is chemically composed of $Al(OH)_x(PO_4)_y$ in which the ratio of hydroxyls to phosphate depends on the precipitation conditions. As a consequence, AP is non-crystalline (amorphous), because the incorporation of phosphate interferes with the crystallization process, and, in contrast to AH, has a negative surface charge at neutral pH (HogenEsch et al. 2018; Powell et al. 2015; He et al. 2015). Higher solubility of AP compared to AH is clearly seen in dissolution experiments with adjuvants in vitro (Seeber et al. 1991; personal unpublished data). Thus, we conclude that our finding is mainly attributed to these physicochemical differences favoring release and dissolution of Al from AP adjuvant.

A further reason for the high recovery of AH-adjuvants 80 days after injection could be the development of granuloma as a foreign body reaction subsequently preventing Al dissolution. Although more commonly seen after SC application of AH-adjuvants, development of persistent granuloma at the injection site has also been reported after IM application, often accompanied by Al contact allergy

(Netterlid et al. 2013). Since IM granuloma is less palpable, occurrence might be underestimated. For example, a 100% frequency of granuloma was observed in the neck of 31 pigs after IM injection of AH-adjuvanted vaccines (Valtulini et al. 2005). Also in mice a high number was found after IM injection of Alhydrogel® or HBV Engerix® vaccine (93% at day 45 decreasing to still 35% at day 270 p.v.; Crépeaux et al. 2015).

The highest estimate of absolute Al release from all injection sites for pAP is fully consistent with the highest increase in plasma AUC and bone Al concentration found for this group. Corresponding correlations obtained for all rats between estimated Al amount released from the injection site and both plasma and bone Al exposure confirm that Al release can be interpreted as systemically available amount and increase in bone and plasma exposure are fairly proportional to this amount. However, we cannot exclude overestimation of systemically available amounts as the total Al release might include a fraction of still undissolved Al particles phagocytosed and transported to the draining lymph node by antigen-presenting immune cells (He et al. 2015).

The highest total bone Al concentration measured in our rats (3.35 µg/g ww) is far below levels of toxicological concern. Studies conducted by Sun et al. (2015, 2016) indicated that rats with bone Al concentrations up to 15 µg/g (ww) were without abnormal findings, whereas above 20 µg/g (ww) bone formation markers decreased and oxidative stress markers increased, and in groups > 30 µg/g (ww) bone mineral density (BMD) decreased significantly.

Also in humans bone Al levels below 10–15 µg/g are not associated with "Al-overload" or any signs of bone toxicity (Klein 2019; Hellström et al. 2005, 2006; Van Landeghem et al. 1998).

Extrapolating the Al increase found in humerus bone to the whole rat skeleton (using 25 g skeleton weight for a 350 g rat [Brown et al. 1997; O'Flaherty 1991)], a mean treatment-related Al amount "added" to the skeleton of 82.6, 7.1, 31.3, and 35.4 µg per rat is estimated for groups pAP, V1, V2, and V3, respectively. These amounts represent 5.3–12.0% of the corresponding total Al amounts released from the injection site (Table 2). These percentages are in line with dose fractions of 3–20% found in rat skeleton during 1 year after a single IV dose of ^{26}Al-chloride (Steinhausen 1997).

Very low brain Al concentrations were observed in all groups. Geometric mean level in the control group (0.12 µg/g ww) was well in line with reported control levels in rat brain of 0.02–0.8 µg/g ww (Ogasawara et al. 2002; Veiga et al. 2013; Lin et al. 2015). Statistical significance of brain Al levels in the vaccine groups is not consistent with the ranking of the products regarding Al release from injection site or Al concentration in bone. Of note, despite its highest bioavailable Al amount and highest increase in bone and plasma Al exposure pAP did not show any increase in Al concentration in brain. From ^{26}Al-kinetic data in rats it is known that in contrast to bone only a very small fraction of dose (< 0.01%) retains in brain (Yokel and McNamara 2001; Walker et al. 1994; Yumoto et al. 1997). Several animal studies demonstrated that brain has much lower Al concentrations than many other tissues, also in normal human beings (Yokel and McNamara 2001). A fraction of 0.01% of the highest bioavailable amount in our study (836.8 µg) would correspond to 0.084 µg Al as the maximum amount supposed to have reached brain. Equal distribution in a rat brain weighing 2 g (estimate for a male 350 g rat; Brown et al. 1997) would lead to a maximum brain concentration increase of 0.042 µg/g ww. Considering our control group mean level (0.13 ± 0.04 µg/g ww), this small difference is unlikely to be detected. Overall, this rather supports the notion that the small increases in brain Al concentration found for V1–V3 are chance findings.

As we determined Al concentration in a whole brain hemisphere Al clusters due to focal accumulation which have been reported for human brain tissues (House et al. 2012) could not be missed. Furthermore, as determination by AAS comprises dissolved Al^{3+} ions as well as insoluble Al species, our results would also capture any Al particles transported into the brain by macrophages which has been postulated by some authors (Gherardi et al. 2015; Crépeaux et al. 2015; Shardlow et al. 2018). Based on our results, we conclude that contribution of such particulate Al amounts, if any, are marginal.

In summary, the present study for the first time revealed systemically available Al from IM injected adjuvants and adjuvanted vaccines in vivo through increase of Al levels mainly in bone. The findings were corroborated by significant correlations with total Al release from the injection site. Moreover, our results clearly indicate that the rate of systemic availability of Al is markedly higher from AP- than from AH-adjuvanted vaccines. We are aware that tissue determination on day 80 is only a cross-sectional view and that different bone levels might only reflect different rates of absorption. This would imply that, once Al absorption is completed, two products with comparable Al doses might reach comparable cumulative Al concentrations in bone, however, at different time points.

Increases of Al exposure in plasma and bone observed in rats cannot one-to-one be translated to humans, this is especially true for bone allometry with inter-species differences in bone architecture and remodeling (Bagi et al. 2011; Barak et al. 2013). In relation to body weight the doses applied to our rats (mean body weight 350 g) were 170 times higher compared to application to a 60 kg human adult. Considering an allometric scaling factor of 6.2 which is usually applied for dose conversion on mg/kg basis between rats and humans in pharmacology (FDA 2005; Nair and Jacob 2018),

this ratio is still 27. Thus, we may expect that after a single vaccination in adults Al levels in bone, and even more valid in plasma and brain, will be indistinguishable from baseline levels. With respect to children simple allometric dose scaling is not adequate, in particular for infants below 2 years of age due to complex age-related developmental changes (Lu and Rosenbaum 2014). For that purpose, physiology-based modeling is required as it is increasingly used in pediatric drug development and toxicologic evaluations (Sharma and McNeill 2009; Barrett et al. 2012). The results of this study will be highly valuable for establishment of a physiology-based toxicokinetic (PBTK) model for Al exposure from adjuvants (Weisser et al. 2017).

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Compliance with ethical standards

Conflict of interest Author Jennifer D. Oduro declares that she is employee at preclinics GmbH, a contract research organization that has received payment for conducting the animal study. All other authors declare that they have no conflict of interest.

Ethical approval All applicable international, national institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (preclinics GmbH, Germany) at which the studies were conducted.

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EXHIBIT 364

Centers for Disease Control and Prevention
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National Center for Health Statistics

Data Table for Boys Length-for-age and Weight-for-age Charts

Boys Length-for-age Percentiles, Birth to 24 Months

Month	L	M	S	2nd (2.3rd)	5th	10th	25th	50th	75th	90th	95th	98th (97.7
0	1	49.8842	0.03795	46.09799	46.77032	47.45809	48.60732	49.8842	51.16108	52.31031	52.99808	53.67
1	1	54.7244	0.03557	50.83131	51.52262	52.2298	53.41147	54.7244	56.03733	57.219	57.92618	58.61
2	1	58.4249	0.03424	54.42396	55.13442	55.8612	57.0756	58.4249	59.7742	60.9886	61.71538	62.42
3	1	61.4292	0.03328	57.34047	58.06652	58.80924	60.0503	61.4292	62.8081	64.04916	64.79188	65.51
4	1	63.886	0.03257	59.72447	60.46344	61.21939	62.48254	63.886	65.28946	66.55261	67.30856	68.04
5	1	65.9026	0.03204	61.67956	62.42946	63.19658	64.4784	65.9026	67.3268	68.60862	69.37574	70.12
6	1	67.6236	0.03165	63.34303	64.10314	64.88071	66.18	67.6236	69.0672	70.36649	71.14406	71.90
7	1	69.1645	0.03139	64.82235	65.5934	66.38216	67.70013	69.1645	70.62887	71.94684	72.7356	73.50
8	1	70.5994	0.03124	66.18835	66.97163	67.77291	69.1118	70.5994	72.087	73.42589	74.22717	75.01
9	1	71.9687	0.03117	67.48217	68.27886	69.09384	70.45564	71.9687	73.48176	74.84356	75.65854	76.45
10	1	73.2812	0.03118	68.71138	69.52286	70.35297	71.74005	73.2812	74.82235	76.20943	77.03954	77.85
11	1	74.5388	0.03125	69.88013	70.70738	71.55363	72.96769	74.5388	76.10991	77.52397	78.37022	79.19
12	1	75.7488	0.03137	70.99632	71.84023	72.70353	74.14605	75.7488	77.35155	78.79407	79.65737	80.50
13	1	76.9186	0.03154	72.06657	72.92816	73.80954	75.28228	76.9186	78.55492	80.02766	80.90904	81.77
14	1	78.0497	0.03174	73.09511	73.97491	74.87492	76.37879	78.0497	79.72061	81.22448	82.12449	83.00
15	1	79.1458	0.03197	74.08522	74.98384	75.9031	77.43914	79.1458	80.85246	82.3885	83.30776	84.20
16	1	80.2113	0.03222	75.04248	75.96033	76.89925	78.46814	80.2113	81.95446	83.52335	84.46227	85.38
17	1	81.2487	0.0325	75.96753	76.90533	77.86466	79.46765	81.2487	83.02975	84.63274	85.59207	86.52
18	1	82.2587	0.03279	76.86417	77.8221	78.80202	80.43942	82.2587	84.07798	85.71538	86.6953	87.65

19	1	83.2418	0.0331	77.73119	78.70973	79.71074	81.38338	83.2418	85.10022	86.77286	87.77387	88.75
20	1	84.1996	0.03342	78.5717	79.57106	80.59338	82.30162	84.1996	86.09758	87.80582	88.82814	89.82
21	1	85.1348	0.03376	79.3865	80.40724	81.45143	83.19621	85.1348	87.07339	88.81817	89.86236	90.88
22	1	86.0477	0.0341	80.17925	81.22133	82.28734	84.06859	86.0477	88.02681	89.80806	90.87407	91.91
23	1	86.941	0.03445	80.95077	82.01447	83.1026	84.92082	86.941	88.96118	90.7794	91.86753	92.93
24	1	87.8161	0.03479	81.70586	82.79087	83.9008	85.75545	87.8161	89.87675	91.7314	92.84133	93.92

Boys Weight-for-age Percentiles, Birth to 24 Months

Month	L	M	S	2nd (2.3rd)	5th	10th	25th	50th	75th	90th	95th
0	0.3487	3.3464	0.14602	2.459312	2.603994	2.757621	3.027282	3.3464	3.686659	4.011499	4.214527
1	0.2297	4.4709	0.13395	3.39089	3.566165	3.752603	4.080792	4.4709	4.889123	5.290726	5.542933
2	0.197	5.5675	0.12385	4.31889	4.522344	4.738362	5.117754	5.5675	6.048448	6.509323	6.798348
3	0.1738	6.3762	0.11727	5.018434	5.240269	5.475519	5.888058	6.3762	6.897306	7.395936	7.708329
4	0.1553	7.0023	0.11316	5.561377	5.797135	6.046988	6.484777	7.0023	7.554286	8.082087	8.412602
5	0.1395	7.5105	0.1108	5.996672	6.244465	6.507016	6.966941	7.5105	8.090161	8.644384	8.991445
6	0.1257	7.934	0.10958	6.352967	6.611702	6.885864	7.366195	7.934	8.539707	9.119041	9.481939
7	0.1134	8.297	0.10902	6.653301	6.922131	7.207057	7.706413	8.297	8.927371	9.530656	9.908738
8	0.1021	8.6151	0.10882	6.913126	7.19127	7.486158	8.003205	8.6151	9.268678	9.894622	10.28713
9	0.0917	8.9014	0.10881	7.144822	7.431644	7.735837	8.26946	8.9014	9.5769	10.22433	10.63055
10	0.082	9.1649	0.10891	7.356558	7.651572	7.964565	8.5139	9.1649	9.861313	10.5293	10.94868
11	0.073	9.4122	0.10906	7.55441	7.857229	8.178615	8.742959	9.4122	10.12867	10.81641	11.24845
12	0.0644	9.6479	0.10925	7.742219	8.052577	8.382077	8.960956	9.6479	10.38387	11.09087	11.53526
13	0.0563	9.8749	0.10949	7.922091	8.239848	8.577324	9.170505	9.8749	10.63014	11.35618	11.81281
14	0.0487	10.0953	0.10976	8.095984	8.421033	8.76637	9.373665	10.0953	10.86959	11.61449	12.08325
15	0.0413	10.3108	0.11007	8.265127	8.597424	8.950586	9.571948	10.3108	11.10416	11.86797	12.34891

16	0.0343	10.5228	0.11041	8.430734	8.770274	9.13126	9.7667	10.5228	11.33528	12.11808	12.61125
17	0.0275	10.7319	0.11079	8.593128	8.939942	9.308795	9.958406	10.7319	11.5637	12.36571	12.87128
18	0.0211	10.9385	0.11119	8.752902	9.107002	9.483736	10.14755	10.9385	11.7897	12.61101	13.12906
19	0.0148	11.143	0.11164	8.909889	9.27136	9.656076	10.33431	11.143	12.01396	12.855	13.38579
20	0.0087	11.3462	0.11211	9.065209	9.434095	9.826848	10.51961	11.3462	12.23713	13.09811	13.64181
21	0.0029	11.5486	0.11261	9.219037	9.595435	9.996335	10.70383	11.5486	12.45983	13.3411	13.89795
22	-0.0028	11.7504	0.11314	9.371554	9.755556	10.16471	10.88716	11.7504	12.6823	13.58426	14.15453
23	-0.0083	11.9514	0.11369	9.522741	9.914417	10.33191	11.06946	11.9514	12.90424	13.82718	14.41108
24	-0.0137	12.1515	0.11426	9.672527	10.07194	10.49784	11.25065	12.1515	13.12555	14.06979	14.66753

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EXHIBIT 365

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

National Center for Health Statistics

Data Table for Girls Length-for-age and Weight-for-age Charts

Girls Length-for-age Percentiles, Birth to 24 Months

Month	L	M	S	2nd (2.3rd)	5th	10th	25th	50th	75th	90th	95th	98 (97
0	1	49.1477	0.0379	45.4223043	46.08383	46.76056	47.89133	49.1477	50.40407	51.53484	52.21157	52.
1	1	53.6872	0.0364	49.7787718	50.4728	51.18277	52.3691	53.6872	55.0053	56.19163	56.9016	57.
2	1	57.0673	0.03568	52.9949775	53.71811	54.45785	55.69393	57.0673	58.44067	59.67675	60.41649	61.
3	1	59.8029	0.0352	55.5927758	56.34038	57.10515	58.38306	59.8029	61.22274	62.50065	63.26542	64.
4	1	62.0899	0.03486	57.7609922	58.52969	59.31604	60.63	62.0899	63.5498	64.86376	65.65011	66.
5	1	64.0301	0.03463	59.5953753	60.38286	61.18844	62.53451	64.0301	65.52569	66.87176	67.67734	68.
6	1	65.7311	0.03448	61.1982833	62.00319	62.82658	64.20243	65.7311	67.25977	68.63562	69.45901	70.
7	1	67.2873	0.03441	62.656588	63.47888	64.32005	65.72562	67.2873	68.84898	70.25455	71.09572	71.
8	1	68.7498	0.0344	64.0198138	64.85973	65.71894	67.15464	68.7498	70.34496	71.78066	72.63987	73.
9	1	70.1435	0.03444	65.3120157	66.16996	67.0476	68.51411	70.1435	71.77289	73.2394	74.11704	74.
10	1	71.4818	0.03452	66.5466965	67.42304	68.31951	69.81746	71.4818	73.14614	74.64409	75.54056	76.
11	1	72.771	0.03464	67.7294251	68.62467	69.54048	71.07075	72.771	74.47125	76.00152	76.91733	77.
12	1	74.015	0.03479	68.8650363	69.77953	70.71503	72.2782	74.015	75.7518	77.31497	78.25047	79.
13	1	75.2176	0.03496	69.9583854	70.89228	71.84762	73.44396	75.2176	76.99124	78.58758	79.54292	80.
14	1	76.3817	0.03514	71.0135941	71.96683	72.94195	74.57133	76.3817	78.19207	79.82145	80.79657	81.
15	1	77.5099	0.03534	72.0315003	73.00432	73.99947	75.66234	77.5099	79.35746	81.02033	82.01548	82.
16	1	78.6055	0.03555	73.016649	74.00908	75.0243	76.72069	78.6055	80.49031	82.1867	83.20192	84.
17	1	79.671	0.03576	73.9729301	74.98475	76.01981	77.74936	79.671	81.59264	83.32219	84.35725	85.
18	1	80.7079	0.03598	74.9001595	75.93146	76.98644	78.74927	80.7079	82.66653	84.42936	85.48434	86.

19	1	81.7182	0.0362	75.8018023	76.8524	77.92712	79.72293	81.7182	83.71347	85.50928	86.584	87.
20	1	82.7036	0.03643	76.6778157	77.74783	78.84242	80.67144	82.7036	84.73576	86.56478	87.65937	88.
21	1	83.6654	0.03666	77.5310529	78.62035	79.73466	81.59662	83.6654	85.73418	87.59614	88.71045	89.
22	1	84.604	0.03688	78.363609	79.47174	80.60531	82.49946	84.604	86.70854	88.60269	89.73626	90.
23	1	85.5202	0.03711	79.1728908	80.3	81.453	83.3796	85.5202	87.6608	89.5874	90.7404	91.
24	1	86.4153	0.03734	79.9618054	81.10777	82.28006	84.23889	86.4153	88.59171	90.55054	91.72283	92.

Girls Weight-for-age Percentiles, Birth to 24 Months

Month	L	M	S	2nd (2.3rd)	5th	10th	25th	50th	75th	90th	95th
0	0.3809	3.2322	0.14171	2.394672	2.532145	2.677725	2.932331	3.2322	3.55035	3.852667	4.040959
1	0.1714	4.1873	0.13724	3.161067	3.326209	3.502477	3.814261	4.1873	4.590075	4.979539	5.225436
2	0.0962	5.1282	0.13	3.941053	4.13172	4.335355	4.695944	5.1282	5.596104	6.049862	6.337067
3	0.0402	5.8458	0.12619	4.53604	4.745935	4.970282	5.368044	5.8458	6.364222	6.868317	7.188096
4	-0.005	6.4237	0.12402	5.013368	5.238858	5.480078	5.90832	6.4237	6.984281	7.530756	7.87815
5	-0.043	6.8985	0.12274	5.403844	5.642267	5.897544	6.351329	6.8985	7.495018	8.077933	8.449225
6	-0.0756	7.297	0.12204	5.729383	5.97888	6.246243	6.72212	7.297	7.925102	8.540297	8.93289
7	-0.1039	7.6422	0.12178	6.008387	6.267836	6.546104	7.042017	7.6422	8.299352	8.94444	9.356859
8	-0.1288	7.9487	0.12181	6.253445	6.522061	6.810403	7.324907	7.9487	8.633118	9.306424	9.737639
9	-0.1507	8.2254	0.12199	6.472906	6.750018	7.047717	7.579535	8.2254	8.935413	9.63531	10.08429
10	-0.17	8.48	0.12223	6.673828	6.958886	7.265345	7.813398	8.48	9.214115	9.939115	10.4049
11	-0.1872	8.7192	0.12247	6.862262	7.15483	7.46957	8.032975	8.7192	9.476145	10.22495	10.7067
12	-0.2024	8.9481	0.12268	7.042612	7.342376	7.665043	8.24313	8.9481	9.726833	10.49835	10.99531
13	-0.2158	9.1699	0.12283	7.217847	7.524538	7.854825	8.446994	9.1699	9.969431	10.76258	11.27401
14	-0.2278	9.387	0.12294	7.389684	7.70313	8.040838	8.646697	9.387	10.20666	11.02071	11.54612
15	-0.2384	9.6008	0.12299	7.559527	7.879566	8.224501	8.843658	9.6008	10.43988	11.27403	11.81285

16	-0.2478	9.8124	0.12303	7.727588	8.054179	8.406286	9.038616	9.8124	10.67062	11.52454	12.07652
17	-0.2562	10.0226	0.12306	7.894535	8.227652	8.586898	9.232317	10.0226	10.89976	11.77319	12.33814
18	-0.2637	10.2315	0.12309	8.060311	8.399952	8.766325	9.424795	10.2315	11.12747	12.02024	12.59804
19	-0.2703	10.4393	0.12315	8.224599	8.570832	8.944403	9.616043	10.4393	11.3542	12.26642	12.85712
20	-0.2762	10.6464	0.12323	8.387882	8.74076	9.121584	9.806487	10.6464	11.58033	12.51209	13.11573
21	-0.2815	10.8534	0.12335	8.55031	8.909946	9.298148	9.996544	10.8534	11.80669	12.75831	13.37511
22	-0.2862	11.0608	0.1235	8.712397	9.078906	9.474611	10.18672	11.0608	12.03376	13.00554	13.6357
23	-0.2903	11.2688	0.12369	8.8741	9.247632	9.651002	10.37713	11.2688	12.26184	13.25422	13.89801
24	-0.2941	11.4775	0.1239	9.035869	9.416516	9.827655	10.56799	11.4775	12.49092	13.50419	14.16181

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EXHIBIT 366

Vaccines and Autoimmunity

Vaccines and Autoimmunity

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Introduction

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Vaccines and Autoimmunity is a result of decades of experience in vaccinology, immunology, and autoimmunity, and of a review of the vast literature in this field. The book has three parts. Part I deals with general mechanisms of vaccine- and adjuvant-induced autoimmunity. In Parts II and III, we have asked the different authors to summarize, on one hand, individual vaccines and which common autoimmune diseases they may trigger in susceptible individuals (Part III), and on the other, the common autoimmune diseases and identified vaccines which may trigger their emergence (Part III).

The editors of this book are quite confident that vaccinations represent one of the most remarkable revolutions in medicine. Indeed, vaccines have been used for over 300 years and are probably one of the most effective strategies for preventing the morbidity and mortality associated with infections. Like other drugs, vaccines can cause adverse events, but unlike conventional drugs, which are prescribed to people who are ill, vaccines are administered to healthy individuals, which increases the concern over adverse reactions. Most side effects attributed to vaccines are mild, acute, and transient. Nonetheless, rare reactions, such as hypersensitivity and induction of autoimmunity, do occur, and can be severe and even fatal. In this regard, the fact that vaccines are delivered to billions of people without preliminary screening for underlying susceptibilities is thus of concern (Bijl *et al.*, 2012; Tomljenovic and Shaw, 2012; Soriano *et al.*, 2014).

Indeed, it is naive to believe that all humans are alike. Notably, autoimmune diseases have been increasingly recognized as having a genetic basis, mediated by HLA subtypes. For instance, celiac disease has been strongly associated with HLA haplotype DR3-DQ2 or DR4-DQ8 (Liu *et al.*, 2014), multiple sclerosis with HLA-DRB1 (Yates *et al.*, 2014), rheumatoid arthritis with HLA-DR4 and HLA-DQ8 (Vassallo *et al.*, 2014), and type I diabetes with HLA-DR3/4 (Steck *et al.*, 2014). Thus, certain HLA genes create a genetic predisposition toward development of autoimmune disease, typically requiring some environmental trigger to evolve into a full-blown disease state (Luckey *et al.*, 2011). One such environmental trigger which is commonly associated with development of autoimmunity is viral (Epstein Barr virus, cytomegalovirus, and hepatitis C virus) or bacterial (*Heliobacter pylori*) challenge (Rose, 2010; Magen and Delgado, 2014).

The multifacet associations between infectious agents and subsequent development of autoimmune or autoinflammatory conditions have been well established, and a number of mechanisms by which infectious agents can bring about such responses have been identified (molecular mimicry, epitope spreading, polyclonal activation, and others) (Molina and Shoenfeld, 2005; Kivity *et al.*, 2009; Shoenfeld, 2009; Rose, 2010).

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Recently, we and others have suggested another mechanism, namely the adjuvant effect, by which infections may relate to autoimmunity in a broader sense (Rose, 2010; Rosenblum *et al.*, 2011; Shoenfeld and Agmon-Levin, 2011; Zivkovic *et al.*, 2012; Perricone *et al.*, 2013). Adjuvants are substances which enhance the immune response. For this purpose, they are routinely included in vaccine formulations, the most common of which are aluminum compounds (alum hydroxide and phosphate). Although the mechanisms of adjuvancy are not fully elucidated, adjuvants seem to modulate a common set of genes, promote antigen-presenting cell recruitment, and mimic specific sets of conserved molecules, such as bacteria components, thus increasing the innate and adaptive immune responses to the injected antigen (Agmon-Levin *et al.*, 2009; Israeli *et al.*, 2009; McKee *et al.*, 2009; Exley *et al.*, 2010; Perricone *et al.*, 2013).

Although the activation of autoimmune mechanisms by both infectious agents and substances with adjuvant properties (such as those found in vaccines) is common, the appearance of an autoimmune disease is not as widespread and apparently not always agent-specific. The adjuvant effect of microbial particles, namely the nonantigenic activation of the innate and regulatory immunity, as well as the expression of various regulatory cytokines, may determine if an autoimmune response remains limited and harmless or evolves into a full-blown disease. Additionally, as already mentioned, the genetic background of an individual may determine the magnitude of adverse manifestations. For example, it has been shown that the vaccine for Lyme disease is capable of triggering arthritis in genetically susceptible hamsters and that, when the adjuvant aluminum hydroxide is added to the vaccine, 100% of the hamsters develop arthritis (Croke *et al.*, 2000). Other studies have shown that the development of inflammatory joint disease and rheumatoid arthritis in adults in response to the HepA and HepB vaccines, respectively, is correlated to the HLA subtype of the vaccinated individual (Ferrazzi *et al.*, 1997; Pope *et al.*, 1998). Given that aluminum works as an adjuvant by increasing expression of MHC (Ulanova *et al.*, 2001), it perhaps should not be surprising that in individuals susceptible to autoimmune disease on the basis of the MHC, HLA subtype might be adversely affected by the use of aluminum hydroxide in vaccines. In addition to aluminum, the vaccine preservative thimerosal has also been demonstrated to induce a systematic autoimmune syndrome in transgenic HLA-DR4 mice (Havarinasab *et al.*, 2004), while mice with a genetic susceptibility for autoimmune disease show profound behavioral and neuropathological disturbances. These results are not observed in strains of mice without autoimmune sensitivity.

We have recently reported a new syndrome: "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA), which encompasses a spectrum of immune-mediated diseases triggered by an adjuvant stimulus such as chronic exposure to silicone, tetramethylpentadecane, pristane, aluminum, and other adjuvants, as well as infectious components, which may also have an adjuvant effect. All these environmental factors have been found to induce autoimmunity and inflammatory manifestations by themselves, both in animal models and in humans (Israeli *et al.*, 2009; Shaw and Petrik, 2009; Shoenfeld and Agmon-Levin, 2011; Gherardi and Authier, 2012; Israeli, 2012; Cruz-Tapias *et al.*, 2013; Lujan *et al.*, 2013; Perricone *et al.*, 2013).

The definition of the ASIA syndrome thus helps to detect those subjects who have developed autoimmune phenomena upon exposure to adjuvants from different sources. For example, the use of medical adjuvants has become common practice, and substances such as aluminum adjuvant are added to most human and animal vaccines, while the adjuvant silicone is extensively used for breast implants and cosmetic procedures (Kaiser *et al.*, 1990; Molina and Shoenfeld, 2005; Israeli *et al.*, 2009; Shoenfeld and Agmon-Levin, 2011; Cohen Tervaert and Kappel, 2013). Furthermore, "hidden adjuvants" such as infectious material and house molds have also been associated with different immune-mediated conditions associated with the so-called "sick-building syndrome" (Israeli and Pardo, 2010; Perricone *et al.*, 2013).

Although ASIA may be labeled a "new syndrome," in reality it reflects old truths given a formal label (Meroni, 2010). Notably, in 1982, compelling evidence from epidemiological, clinical, and animal research emerged to show that Guillain-Barre syndrome and other demyelinating autoimmune neuropathies (i.e., acute disseminated encephalomyelitis and multiple sclerosis) could occur up to 10 months following vaccination (Poser and Behan, 1982). In such cases, the disease would first manifest with vague symptoms (arthralgia, myalgia, paraesthesia, weakness; all of which are typical ASIA symptoms), which were frequently deemed insignificant and thus ignored by the treating physicians. However, these

symptoms would progress slowly and insidiously until the patient was exposed to a secondary immune stimulus (in the form of either infection or vaccination). This would then trigger the rapid and acute clinical manifestation of the disease (Poser and Behan, 1982). In other words, it was the secondary anamnestic response that would bring about the acute overt manifestation of an already present subclinical long-term persisting disease.

Thus, it was already recognized in the early 1980s that vaccine-related manifestations often presented themselves as unspecific, yet clinically relevant symptoms (termed "bridging symptoms" Poser and Behan (1982) or "nonspecific ASIA symptoms" by us (Shoenfeld and Agmon-Levin, 2011)). These manifestations pointed to a subclinical, slowly evolving disease. Whether this disease would eventually progress to its full-blown clinically apparent form depended on whether the individual was further exposed to noxious immune stimuli, including subsequent vaccinations. As a case in point, we recently described six cases of systemic lupus following HPV vaccination (Gatto *et al.*, 2013). In all six cases, several common features were observed; namely, a personal or familial susceptibility to autoimmunity and an adverse response to a prior dose of the vaccine, both of which were associated with a higher risk of post-vaccination full-blown autoimmunity. Similarly, in an analysis of 93 cases of autoimmunity following hepatitis B vaccination (Zafrir *et al.*, 2012), we identified two major susceptibility factors: (i) exacerbation of adverse symptoms following additional doses of the vaccine (47% of patients); and (ii) personal and familial history of autoimmunity (21%).

It should further be noted that some individuals who are adversely afflicted through exposure to adjuvants do not satisfy all of the criteria that are necessary to diagnose a full-blown and clinically apparent autoimmune disease (Perricone *et al.*, 2013). Nonetheless, these individuals are at higher risk of developing full-blown autoimmunity following subsequent adjuvant exposure, whether that be via infections or vaccinations (Poser and Behan, 1982; Zafrir *et al.*, 2012; Gatto *et al.*, 2013).

A casual glance at the US Centers for Disease Control and Prevention (CDC, 2013)_immunization schedule for infants shows that according to the US prescribed guidelines, children receive up to 19 vaccinations during infancy, many of which are multivalent in the first 6 months of their life (Table I.1).

The various vaccines given to children, as well as adults, may contain either whole weakened infectious agents or synthetic peptides and genetically engineered antigens of infectious agents and adjuvants (typically aluminum). In addition, they also contain diluents, preservatives (thimerosal, formaldehyde), detergents (polysorbate), and residuals of culture growth media (*Saccharomyces cerevisiae*, gelatin, bovine extract, monkey kidney tissue, etc.; Table I.2). The safety of these residuals has not been thoroughly investigated, primarily because they are presumed to be present only in trace amounts following the vaccine manufacture purification process. However, some studies

Table I.1 Typical pediatric vaccine schedule for preschool children currently recommended by the US Centers for Disease Control and Prevention (2013a). Shaded boxes indicate the age range in which the vaccine can be given. Asterisks denote Al-adjuvanted vaccines. Hep A is given in 2 doses spaced at least 6 months apart. According to this schedule, by the time a child is 2 years of age, they would have received 27 vaccinations (3 × HepB, 3 × Rota, 4 × DTaP, 4 × Hib, 4 × PCV, 3 × IPV, 2 × Influenza, 1 × MMR, 1 × Varicella, and 2 × HepA)

Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
HepB*	HepB*			HepB*						
	Rota	Rota	Rota							
	DTaP*	DTaP*	DTaP*		DTaP*			DTaP*		
	Hib*	Hib*	Hib*	Hib*						
	PCV*	PCV*	PCV*	PCV*						
	IPV	IPV		IPV					IPV	
				Influenza (yearly)						
				MMR				MMR		
				Varicella				Varicella		
					HepA*					

Hep A, hepatitis A; Hep B, hepatitis B; Rota, rotavirus; DTaP, diphtheria-pertussis-tetanus; Hib, *Haemophilus influenzae* type b; PCV, pneumococcal; IPV, inactivated polio; MMR, measles-mumps-rubella

Table I.2 Complete list of vaccine ingredients (i.e., adjuvants and preservatives) and substances used during the manufacture of commonly used vaccines. Adapted from US Centers for Disease Control and Prevention (2013b)

Vaccine	Vaccine excipient and media summary
DT (Sanofi)	aluminum potassium sulfate, peptone, bovine extract, formaldehyde, thimerosal (trace), modified Mueller and Miller medium
DTaP (Daptacel)	aluminum phosphate, formaldehyde, glutaraldehyde, 2-phenoxyethanol, Stainer–Scholte medium, modified Mueller's growth medium, modified Mueller–Miller casamino acid medium (without beef heart infusion)
DTaP (Infanrix)	formaldehyde, glutaraldehyde, aluminum hydroxide, polysorbate 80, Fenton medium (containing bovine extract), modified Latham medium (derived from bovine casein), modified Stainer–Scholte liquid medium
DTaP (Tripedia)	sodium phosphate, peptone, bovine extract (US sourced), formaldehyde, ammonium sulfate, aluminum potassium sulfate, thimerosal (trace), gelatin, polysorbate 80 (Tween 80), modified Mueller and Miller medium, modified Stainer–Scholte medium
DTaP-HepB-IPV (Pediarix)	formaldehyde, gluteraldehyde, aluminum hydroxide, aluminum phosphate, lactalbumin hydrolysate, polysorbate 80, neomycin sulfate, polymyxin B, yeast protein, calf serum, Fenton medium (containing bovine extract), modified Latham medium (derived from bovine casein), modified Stainer-Scholte liquid medium, Vero (monkey kidney) cells
DTaP-IPV/Hib (Pentacel)	aluminum phosphate, polysorbate 80, formaldehyde, gutaraldehyde, bovine serum albumin, 2-phenoxethanol, neomycin, polymyxin B sulfate, Mueller's Growth Medium, Mueller–Miller casamino acid medium (without beef heart infusion), Stainer–Scholte medium (modified by the addition of casamino acids and dimethyl-beta-cyclodextrin), MRC-5 (human diploid) cells, CMRL 1969 medium (supplemented with calf serum)
Hib (ActHIB)	ammonium sulfate, formalin, sucrose, Modified Mueller and Miller medium
Hib (Hiberix)	formaldehyde, lactose
Hib (PedvaxHIB)	aluminum hydroxphosphate sulfate
Hib/Hep B (Comvax)	yeast (vaccine contains no detectable yeast DNA), nicotinamide adenine dinucleotide, hemin chloride, soy peptone, dextrose, mineral salts, amino acids, formaldehyde, potassium aluminum sulfate, amorphous aluminum hydroxyphosphate sulfate, sodium borate
Hep A (Havrix)	aluminum hydroxide, amino acid supplement, polysorbate 20, formalin, neomycin sulfate, MRC-5 cellular proteins
Hep A (Vaqta)	amorphous aluminum hydroxyphosphate sulfate, bovine albumin, formaldehyde, neomycin, sodium borate, MRC-5 (human diploid) cells
Hep B (Engerix-B)	aluminum hydroxide, yeast protein, phosphate buffers
Hep B (Recombivax)	yeast protein, soy peptone, dextrose, amino acids, mineral salts, potassium aluminum sulfate, amorphous aluminum hydroxyphosphate sulfate, formaldehyde
Hep A/Hep B (Twinrix)	formalin, yeast protein, aluminum phosphate, aluminum hydroxide, amino acids, phosphate buffer, polysorbate 20, neomycin sulfate, MRC-5 human diploid cells
Human Papillomavirus (HPV) (Cerverix)	vitamins, amino acids, lipids, mineral salts, aluminum hydroxide, sodium dihydrogen phosphate dehydrate, insect cell and viral protein
Human Papillomavirus (HPV) (Gardasil)	yeast protein, vitamins, amino acids, mineral salts, carbohydrates, amorphous aluminum hydroxyphosphate sulfate, L-histidine, polysorbate 80, sodium borate
Influenza (Afluria)	beta-propiolactone, thimerosol (multi-dose vials only), monobasic sodium phosphate, dibasic sodium phosphate, monobasic potassium phosphate, potassium chloride, calcium chloride, sodium taurodeoxycholate, neomycin sulfate, polymyxin B, egg protein
Influenza (Fluarix)	sodium deoxycholate, formaldehyde, octoxynol-10 (Triton X-100), α-tocopheryl hydrogen succinate, polysorbate 80 (Tween 80), hydrocortisone, gentamicin sulfate, ovalbumin
Influenza (Fluvirin)	nonylphenol ethoxylate, thimerosal (multidose vial–trace only in prefilled syringe), polymyxin, neomycin, beta-propiolactone, egg proteins
Influenza (Flulaval)	thimerosal, α-tocopheryl hydrogen succinate, polysorbate 80, formaldehyde, sodium deoxycholate, ovalbumin
Influenza (Fluzone: standard, high-dose, & intradermal)	formaldehyde, octylphenol ethoxylate (Triton X-100), sodium phosphate, gelatin (standard formulation only), thimerosal (multidose vial only), egg protein
Influenza (FluMist)	ethylene diamine tetraacetic acid (EDTA), monosodium glutamate, hydrolyzed porcine gelatin, arginine, sucrose, dibasic potassium phosphate, monobasic potassium phosphate, gentamicin sulfate, egg protein

Table I.2 (*Continued*)

Vaccine	Vaccine excipient and media summary
Meningococcal (MCV4Menactra)	formaldehyde, phosphate buffers, Mueller Hinton agar, Watson Scherp media, Modified Mueller and Miller medium
Meningococcal (MCV4Menveo)	formaldehyde, amino acids, yeast extract, Franz complete medium
Meningococcal (MPSV4Menomune)	thimerosal (multidose vial only), lactose, Mueller Hinton agar, Watson Scherp media
MMR (MMR-II)	vitamins, amino acids, fetal bovine serum, sucrose, sodium phosphate, glutamate, recombinant human albumin, neomycin, sorbitol, hydrolyzed gelatin, chick embryo cell culture, WI-38 human diploid lung fibroblasts
MMRV (ProQuad)	sucrose, hydrolyzed gelatin, sorbitol, monosodium L-glutamate, sodium phosphate dibasic, human albumin, sodium bicarbonate, potassium phosphate monobasic, potassium chloride, potassium phosphate dibasic, neomycin, bovine calf serum, chick embryo cell culture, WI-38 human diploid lung fibroblasts, MRC-5 cells
Pneumococcal (PCV13 – Prevnar 13)	casamino acids, yeast, ammonium sulfate, Polysorbate 80, succinate buffer, aluminum phosphate
Polio (IPV – Ipol)	2-phenoxyethanol, formaldehyde, neomycin, streptomycin, polymyxin B, monkey kidney cells, Eagle MEM modified medium, calf serum protein
Rabies (Imovax)	albumin, neomycin sulfate, phenol, MRC-5 human diploid cells
Rabies (RabAvert)	β-propiolactone, potassium glutamate, chicken protein, ovalbumin, neomycin, chlortetracycline, amphotericin B, human serum albumin, polygeline (processed bovine 14 gelatin)
Rotavirus (RotaTeq)	sucrose, sodium citrate, sodium phosphate monobasic monohydrate, sodium hydroxide, polysorbate 80, cell culture media, fetal bovine serum, vero cells (DNA from porcine circoviruses (PCV) 1 and 2 has been detected in RotaTeq; PCV-1 and PCV-2 are not known to cause disease in humans)
Rotavirus (Rotarix)	amino acids, dextran, sorbitol, sucrose, calcium carbonate, xanthan, Dulbecco's Modified Eagle Medium (DMEM) (Porcine circovirus type 1 (PCV-1) is present in Rotarix; PCV-1 is not known to cause disease in humans)
Td (Decavac)	aluminum potassium sulfate, peptone, formaldehyde, thimerosal, bovine muscle tissue (US sourced), Mueller and Miller medium
Td (Tenivac)	aluminum phosphate, formaldehyde, modified Mueller–Miller casamino acid medium without beef heart infusion
Td (Mass Biologics)	aluminum phosphate, formaldehyde, thimerosal (trace), ammonium phosphate, modified Mueller's media (containing bovine extracts)
Tdap (Adacel)	aluminum phosphate, formaldehyde, glutaraldehyde, 2-phenoxyethanol, ammonium sulfate, Mueller's growth medium, Mueller–Miller casamino acid medium (without beef heart infusion)
Tdap (Boostrix)	formaldehyde, glutaraldehyde, aluminum hydroxide, polysorbate 80 (Tween 80), Latham medium derived from bovine casein, Fenton medium containing a bovine extract, Stainer–Scholte liquid medium
Typhoid (inactivated – Typhim Vi)	hexadecyltrimethylammonium bromide, phenol, polydimethylsiloxane, disodium phosphate, monosodium phosphate
Typhoid (oral – Ty21a)	yeast extract, casein, dextrose, galactose, sucrose, ascorbic acid, amino acids
Varicella (Varivax)	sucrose, phosphate, glutamate, gelatin, monosodium L-glutamate, sodium phosphate dibasic, potassium phosphate monobasic, potassium chloride, sodium phosphate monobasic, EDTA, residual components of MRC-5 cells including DNA and protein, neomycin, fetal bovine serum, human diploid cell cultures
Yellow Fever (YF-Vax)	sorbitol, gelatin, egg protein
Zoster (Shingles – Zostavax)	sucrose, hydrolyzed porcine gelatin, monosodium L-glutamate, sodium phosphate dibasic, potassium phosphate monobasic, neomycin, potassium chloride, residual components of MRC-5 cells including DNA and protein, bovine calf serum

suggest that even these trace amounts may not be inherently safe, as was previously assumed (Moghaddam *et al.*, 2006; Rinaldi *et al.*, 2013).

What is obvious, nonetheless, is that a typical vaccine formulation contains all the necessary biochemical components to induce autoimmune manifestations. With that in mind, our major aim is to inform the medical community regarding the various autoimmune risks associated with different vaccines. Physicians need to be aware that in certain individuals, vaccinations can trigger serious and potentially disabling and even fatal autoimmune manifestations. This is not to say that we oppose vaccination, as it is indeed an important tool of preventative medicine. However, given the fact that vaccines are predominantly administered to previously healthy individuals, efforts should be made to identify those subjects who may be at more risk of developing adverse autoimmune events following vaccine exposure. In addition, careful assessment should be made regarding further vaccine administration in individuals with previous histories of adverse reactions to vaccinations. The necessity of multiple vaccinations over a short period of time should also be considered, as the enhanced adjuvant-like effect of multiple vaccinations heightens the risk of post-vaccine-associated adverse autoimmune and inflammatory manifestations (Tsumiyama *et al.*, 2009; Lujan *et al.*, 2013). Finally, we wish to encourage efforts toward developing safer vaccines, which should be pursued by the vaccine manufacturing industry.

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EXHIBIT 367

TOXICOLOGICAL PROFILE FOR MERCURY

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

March 1999

UPDATE STATEMENT

A Toxicological Profile for Mercury–Draft for Public Comment was released in September 1997. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE, E-29
Atlanta, Georgia 30333

freshwater and saltwater fish and marine mammals to levels that are many times greater than levels in the surrounding water (see Section 1.2).

Mercury is mined as cinnabar ore, which contains mercuric sulfide. The metallic form is refined from mercuric sulfide ore by heating the ore to temperatures above 1,000 degrees Fahrenheit. This vaporizes the mercury in the ore, and the vapors are then captured and cooled to form the liquid metal mercury. There are many different uses for liquid metallic mercury. It is used in producing of chlorine gas and caustic soda, and in extracting gold from ore or articles that contain gold. It is also used in thermometers, barometers, batteries, and electrical switches. Silver-colored dental fillings typically contain about 50% metallic mercury. Metallic mercury is still used in some herbal or religious remedies in Latin America and Asia, and in rituals or spiritual practices in some Latin American and Caribbean religions such as Voodoo, Santeria, and Espiritismo. These uses may pose a health risk from exposure to mercury both for the user and for others who may be exposed to mercury vapors in contaminated air.

Some inorganic mercury compounds are used as fungicides. Inorganic salts of mercury, including ammoniated mercuric chloride and mercuric iodide, have been used in skin-lightening creams. Mercuric chloride is a topical antiseptic or disinfectant agent. In the past, mercurous chloride was widely used in medicinal products including laxatives, worming medications, and teething powders. It has since been replaced by safer and more effective agents. Other chemicals containing mercury are still used as antibacterials. These products include mercurochrome (contains a small amount of mercury, 2%), and thimerosal and phenylmercuric nitrate, which are used in small amounts as preservatives in some prescription and over-the-counter medicines. Mercuric sulfide and mercuric oxide may be used to color paints, and mercuric sulfide is one of the red coloring agents used in tattoo dyes.

Methylmercury is produced primarily by microorganisms (bacteria and fungi) in the environment, rather than by human activity. Until the 1970s, methylmercury and ethylmercury compounds were used to protect seed grains from fungal infections. Once the adverse health effects of methylmercury were known, the use of methymercury- and ethylmercury as fungicides was

levels of methylmercury are of concern, and these are discussed in Section 1.7 of this toxicological profile.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO MERCURY?

If your doctor finds that you have been exposed to significant amounts of mercury, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate.

Children may be exposed to metallic mercury if they play with it. Metallic mercury is a heavy, shiny, silver liquid. When metallic mercury is spilled, it forms little balls or beads. Children are sometimes exposed to metallic mercury when they find it in abandoned warehouses or closed factories, and then play with it or pass it around to friends. Children have also taken metallic mercury from school chemistry and physics labs. Broken thermometers and some electrical switches are other sources of metallic mercury. Sometimes children find containers of metallic mercury that were improperly disposed of, or adults may bring home metallic mercury from work, not knowing that it is dangerous.

To protect your children from metallic mercury, teach them not to play with shiny, silver liquids. Schoolteachers (particularly science teachers) and school staff need to know about students' fascination with metallic mercury. Teachers and school staff should teach children about the dangers of getting sick from playing with mercury, and they should keep metallic mercury in a safe and secured area (such as a closed container in a locked storage room) so that children do not have access to it without the supervision of a teacher. Metallic mercury evaporates slowly, and if it is not stored in a closed container, children may breathe toxic mercury vapors.

In the past, mercurous chloride was widely used in medicinal products such as laxatives, worming medications, and teething powders. These older medicines should be properly disposed of and replaced with safer and more effective medicines. Other chemicals containing mercury, such as mercurochrome and thimerosal (sold as Merthiolate and other brands), are still used as antiseptics or as preservatives in eye drops, eye ointments, nasal sprays, and vaccines. Some skin-lightening

EXHIBIT 368

CURRENT OPINION

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Thiomersal in Vaccines

Balancing the Risk of Adverse Effects with the Risk of Vaccine-Preventable Disease

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Abstract

A number of affluent countries are moving to eliminate thiomersal (thimerosal), an ethylmercury preservative, from vaccines as a precautionary measure because of concerns about the potential adverse effects of mercury in infants. The WHO advocates continued use of thiomersal-containing vaccines in developing countries because of their effectiveness, safety, low cost, wide availability and logistical suitability in this setting.

The guidelines for long-term mercury exposure should not be used for evaluating risk from intermittent single day exposures, such as immunisation using thiomersal-containing vaccines. Similar or higher mercury exposures likely occur from breast feeding and the health benefit of eliminating thiomersal from a vaccine, if any, is likely to be very small. On the other hand, the benefits accrued from the use of thiomersal-containing vaccines are considerably greater but vary substantially between affluent and developing regions of the world. Because of the contribution to overall mercury exposure from breast milk and diet in later life, the removal of thiomersal from vaccines would produce no more than a 50% reduction of mercury exposure in infancy and <1% reduction over a lifetime.

Different public policy decisions are appropriate in different settings to achieve the lowest net risk, viewed from the perspectives of the individual vaccinee or on a population basis. In developing regions of the world, at least over the next decade, far more benefit will accrue from protecting children against widely prevalent vaccine-preventable diseases by focusing efforts aimed at improving infant immunisation uptake by using current, inexpensive, domestically-manufactured, thiomersal-containing vaccines, than by investing in thiomersal-free alternatives.

Mercury is a naturally occurring element to which all humans are exposed.[1-6] It is estimated that natural degassing of the earth's crust is responsible for over half of atmospheric mercury emissions (about 2700–6000 tons per year), whereas anthropogenic releases may account for an additional 2000–3000 tons per year.[7] The serious health consequences of short or long-term, high-dose, dietary-organic mercury exposure, which primarily involved the nervous system, were recognised following investigation of outbreaks of illness from the consumption of contaminated fish in Minamata, Japan and contaminated bread in Iraq, during the last century.[1,8-10]

Current questions regarding mercury toxicity are focused on possible neurological adverse effects

at much lower exposure levels. Although exposure to some level of mercury is universal, quantitative assessment has shown that the three largest contributors of mercury exposure to the general population are diet (primarily fish), dental amalgam and some pharmacological products, such as thiomersal (thimerosal)-containing vaccines.[1,5,8,11-13] Thiomersal is an ethylmercury-containing compound and has been used for decades as a preservative in many vaccines.

Since 1999, two different approaches have been followed regarding thiomersal-containing vaccines for childhood immunisation programmes. The US, countries in the European Union (EU) and a few other affluent countries have implemented measures to eliminate childhood exposure to vaccine-derived thiomersal.[14-21] As of 2004, none of the routine single or multivalent vaccines recommended and routinely used to protect preschool children in the EU or US contain thiomersal.[21-23] Elimination of thiomersal from routine childhood immunisation programmes in these jurisdictions has been achieved essentially by exclusive use of single dose, preservative-free vaccine formats.

However, most countries continue to use thiomersal-containing vaccines in their childhood programmes. The WHO continues to endorse using thiomersal-containing vaccines for children, including malnourished, premature or low-birthweight infants.[24-26] The basis of WHO's position is that pharmacokinetic and epidemiological studies have not demonstrated convincing evidence of ethylmercury toxicity from exposure to thiomersal-containing vaccines, whereas use of these vaccines, particularly in regions of high disease burden, has proven highly effective in protecting children.[27,28]

The two different approaches to the issue of thiomersal in vaccines continue to generate confusion among parents, adult vaccine recipients and healthcare workers who administer vaccines. Even in jurisdictions where thiomersal has been eliminated from vaccines routinely administered to infants, there remain thiomersal-containing vaccines that may be recommended for some high-risk children (e.g. vaccines against influenza, Lyme disease, invasive pneumococcal disease or rabies).

This article reviews evidence of mercury-related health effects from exposure to thiomersal-containing vaccines in the context of other mercury exposures during infancy and over a lifetime, and assess the potential impact of eliminating thiomersal from vaccines. It also examines the public policy implications of eliminating thiomersal from vaccines as a trade-off between two competing risks (i.e. the risk of potential adverse effects attributable to exposure to thiomersal in vaccines versus the risk of vaccine-preventable illness or death), viewed from individual and population health perspectives.

To obtain relevant papers for this review, an electronic search was undertaken of literature published up to March 2004 using Medline (PubMed). Papers subsequently published up to December 2004 were included during the review of the final proofs. An initial Boolean search strategy using the key words 'thimerosal', 'ethylmercury', 'methylmercury', 'neurodevelopmental disorders', 'adverse effects' and 'autism' was utilised. No electronic search limitations were applied. Additional citations were identified through a PubMed search of related articles and from secondary sources cited in primary references.

1. Quantifying Vaccine-Derived Ethylmercury Exposure

In 1999, it was estimated that American infants could receive a cumulative dose of vaccine-derived ethylmercury as high as 187.5µg during the first 6 months of life and up to 237.5µg ethylmercury by 2 years of age.[29] Exposures for some children at high risk who also received influenza vaccine could have been as high as 200µg and 275µg at 6 months and 2 years of age, respectively. Although exposure of American (and most Western European) infants to thiomersal-containing vaccines used in routine immunisation has since been eliminated, several thiomersal-containing vaccines continue to be used for routinely recommended adult immunisations, which could potentially expose persons to a cumulative 950µg of ethylmercury over a lifetime (table I).

The immunisation schedule for infants recommended by the WHO Expanded Programme on Immunisation (EPI) (table II), representing the core of immunisation programmes in most developing countries, potentially exposes most of the world's children to a level of 112.5µg ethylmercury by 14 weeks of age. *Haemophilus influenzae* type b (Hib)

Table I. Vaccine-derived ethylmercury exposure in US Centers for Disease Control immunisation schedules for children, adolescents and adults[30,31]

Age	Vaccines	Ethylmercury dose (μg)	
		per dose	cumulative to 80 years
4–6 years	DTaP, IPV-4, MMR-2		
11–12 years	Td	25	25
Every 10 years	Td	25	150
Annually from age 50 years	Influenza	25	750
Age 65 years	Pneumococcal	25	25
Total to age 80			**950**

DTaP = diphtheria-tetanus-acellular pertussis; **IPV** = inactivated poliovirus vaccine; **MMR** = measles-mumps-rubella; **Td** = tetanus-diphtheria.

vaccine is gradually being introduced into childhood programmes in the developing world,[32] with three doses of Hib vaccine contributing an additional 75μg of ethylmercury in the first 6 months of life. Current EPI recommendations for routine immunisation of adults focus on preventing perinatal tetanus through immunisation of women of childbearing age with tetanus toxoid (table III).[32]

2. Is There Evidence of Adverse Health Effects from Vaccine-Derived Ethylmercury?

A retrospective cohort study first reported in 2001 by the US Institute of Medicine, Immunization Safety Review Committee, suggested a possible association between vaccine-derived ethylmercury and adverse health effects.[29] This study examined up to 7 years of data for children who were enrolled in three US health maintenance organisations (HMOs). The data analysed were from the Vaccine Safety Datalink, which includes vaccination, clinic, hospital discharge and demographic data from seven US HMOs. Results were inconsistent between HMOs and inconclusive, with weak associations (relative risks <2 per 12.5μg increment in ethylmercury) identified between various cumulative exposures to thiomersal and some neurodevelopmental diagnoses, such as speech delay and attention deficit disorder but not autism. No consistent dose-response relationship was detected.

More recently, published studies examining autism and thiomersal-containing vaccines provide evidence that is not consistent with a causal relationship.[33,34] Madsen et al.[33] analysed a national diagnostic registry to assess the incidence of autism in Danish children aged 2–10 years between 1971 and 2000. There was no trend towards increased autism incidence during the period up to 1992 (when thiomersal-containing vaccines were used for childhood immunisations in Denmark). Autism incidence began to rise in 1991 and continued to rise, even after Denmark switched to thiomersal-free vaccines for childhood immunisations during 1992. Autism incidence peaked in 1999, with the highest age-stratified rates among children born between 1993 and 1997. Stehr-Green et al.[34] compared estimated prevalence or reported incidence of childhood au-

Table II. Vaccine-derived ethylmercury exposure for infants immunised as recommended by the WHO Expanded Programme on Immunisation schedule[27]

Age	Vaccines	Hepatitis B vaccine[a]		Ethylmercury dose (μg)	
		scheme 1	scheme 2	scheme 1	scheme 2
Birth	BCG, OPV-0	HBV-1		12.5	
6 weeks	DTP-1, OPV-1, (Hib-1)	HBV-2	HBV-1	37.5 (62.5)[b]	37.5 (62.5)
10 weeks	DTP-2, OPV-2, (Hib-2)		HBV-2	25 (50)	37.5 (62.5)
14 weeks	DTP-3, OPV-3, (Hib-3)	HBV-3	HBV-3	37.5 (62.5)	37.5 (62.5)
9 months	Measles, yellow fever[c]				
Total				**112.5 (187.5)**	**112.5 (187.5)**

a In countries with hepatitis B surface antigen prevalence ≥2%; scheme 1 is recommended in countries with a high risk of perinatal transmission.

b Parentheses indicate ethylmercury dose if the Hib vaccine is also included in the immunisation schedule.

c In countries where yellow fever poses a risk.

BCG = bacilli Calmette-Guérin; **DTP** = diphtheria-tetanus-(whole cell) pertussis; **HBV** = hepatitis B vaccine; **Hib** = *Haemophilus influenzae* type b; **OPV** = oral poliovirus vaccine.

Table III. Vaccine-derived ethylmercury exposure for women of childbearing age (especially pregnant women) immunised as recommended by the WHO Expanded Programme on Immunisation schedule[27]

Vaccine	Timing	Ethylmercury dose (μg)
TT-1	As soon as possible in pregnancy or as early as possible in childbearing years	25
TT-2	At least 4 weeks after TT-1	25
TT-3	At least 6 months after TT-2	25
TT-4 and TT-5	At least 1 year after previous TT dose	50
Total		**125**

TT = tetanus toxoid.

tism with average cumulative doses of vaccine-derived ethylmercury for birth cohorts between the mid-1980s to late 1990s in California, Sweden and Denmark. Although reported incidence (in Sweden and Denmark) and estimated prevalence (in California) of autism-like disorders rose in the late 1980s and accelerated in the early 1990s, the average cumulative vaccine-derived ethylmercury exposures decreased and were eventually eliminated over this period in Sweden and Denmark, while increasing in the US.

In May 2004, the US Institute of Medicine, Immunization Safety Review Committee, investigating whether thiomersal-containing vaccines cause autism, concluded that the body of epidemiological evidence favours rejection of a causal relationship.[35] Subsequently, two published cohort studies in the UK also found no convincing evidence that vaccine-derived thimerosal exposure causes neurodevelopmental disorders. Heron et al.[36] used data from a population-based cohort study of approximately 14 000 children born in 1991 and 1992 from southwest England. Ethylmercury exposure from thiomersal-containing vaccines administered up to 6 months of age was determined from public health immunisation records and analysed against a range of behavioural, speech and motor development criteria that were assessed using validated questionnaires administered to mothers at six specified periods until children were 91 months old. Information on potential confounders (infant gestation birth weight, gender, breastfeeding status and maternal parity, ethnicity, smoking status, education, housing and fish consumption during third trimester) was also collected. Adjusted analyses were most consistent with there being no neurological or behavioural adverse outcome associated with vaccine-derived thiomersal. Andrews et al.[37] retrospectively collected and analysed data on approximately 100 000 term and 2500 pre-term children born in the UK between 1988 and 1997 who had at least 2 years follow-up by general practitioners who were registered with, and contributed to, a research database. Vaccine-derived ethylmercury exposures at 3 and 4 months of age were determined from the research database and analysed against a range of recorded outcome events including neurodevelopmental disorders, autism, problems with behaviour, speech or language, attention deficit disorder, encopresis, enuresis and tics. Potential confounders were not considered. Investigators found no evidence in either term or pre-term children of an association with any of the outcome events, except possibly tics (with which Heron et al.[36] detected no evidence of association).

3. Are Adverse Health Effects from Vaccine-Derived Ethylmercury Plausible?

Plausibility of a possible association between vaccine-derived ethylmercury exposure and mercury-related health effects has been based on the following:

- Presumed similarities in pharmacokinetics and toxicological effects of ethyl- and methylmercury.
- Hypersensitivity reactions after low-dose exposures to thiomersal-containing products.
- Measurable increases in blood mercury following immunisation of infants with thiomersal-containing vaccines.
- Evidence of a dose-response effect from high-dose, acute and chronic occupational and dietary exposures to ethylmercury.

3.1 Are the Pharmacokinetics of Ethyl- and Methylmercury Comparable?

The pharmacokinetics and toxicology of methylmercury have been studied far more extensively than for ethylmercury.[5,29,38] Similar pharmacokinetics and toxicology at lower doses were initially postulated because of their related chemical structures and similar health effects at higher doses.[8,12,15,39] Current regulatory guidelines for organic mercury exposure have therefore been essentially based on the properties and toxicology of methylmercury.[40-43]

The metabolism and toxicological mechanisms of action of ethyl- and methylmercury are complex,[29] and significant differences in the pharmacokinetics between these two compounds are being recognised.[11,12,44,45] Two important differences are the significantly shorter half-life of ethylmercury in blood and less movement of ethylmercury through the blood-brain barrier into the central nervous system.[11,46,47] Magos[46] estimated an allometrically corrected half-life of 18 days for mercury administered as thiomersal, which was within 10% of the measured blood mercury levels reported by Pichichero et al.[11] Data presented by Magos indicate that transient accumulation of blood mercury still results from vaccination with thiomersal-containing vaccines at the 4–6 weeks dose intervals recommended by the WHO/EPI routine infant immunisation schedule for developing countries, although it is significantly less than the estimates based on the longer half-life of methylmercury.[12,46] At the longer 6–8 week primary immunisation dose intervals typically recommended for infants in developed countries, no significant accumulation in blood mercury occurs.[46,47]

3.2 Are the Toxicological Effects of Early Methylmercury Exposure Relevant to Ethylmercury?

Long-term, prospective population-based studies of long-term, low-dose prenatal and dietary mercury exposure to children in the Seychelles Islands,[48,49] Faeroe Islands,[50,51] and New Zealand[52] are based on methylmercury intake. In the Seychelles, chronic, low-dose *in utero* mercury exposures result from mothers eating a predominantly fish-based diet, whereas the fish consumption of Faeroes mothers intermittently changes to the consumption of the meat and blubber of pilot whales.

The Seychelles study,[49] which used maternal and child hair to evaluate prenatal and childhood mercury exposure, respectively, and primarily global neuropsychiatric scales to assess outcome, found no neurological impairment among children up to 9 years of age. The Faeroe Islands study,[51] which used umbilical cord blood and child hair to evaluate prenatal and postnatal mercury exposure, respectively, and domain-specific neuropsychiatric testing to assess outcome, reported subtle neurological deficits in memory, attention and language scores among the 7-year-old children tested. Postnatal mercury exposure was less predictive of these effects than prenatal exposure. Infant neurodevelopment test results have not consistently been shown to predict later dysfunction.[53,54]

The New Zealand study[52] correlated prenatal methylmercury exposure, estimated from analysis of maternal hair samples collected during pregnancy, with scholastic and psychological test results in 6- and 7-year-old children. A possible subtle mercury effect was detected but only after excluding one 'outlier' infant-mother pair from the analysis because of a maternal hair mercury level of 86 mg/kg, which was more than four times that of the other mothers.

Mercury exposure of infants to thiomersal-containing vaccines differs from exposures in the Seychelles, Faeroes and New Zealand studies in several key respects. First, ethylmercury is less neurotoxic than methylmercury. Second, the timing of the exposure is different with only postnatal exposure associated with infant vaccination. Third, the route of exposure (parenteral vs oral) is different and fourth, the exposure from vaccination is intermittent rather than more continuous as in the Seychelles, Faeroes and New Zealand studies.[15,29,55]

3.3 Hypersensitivity Reactions after Low-Dose Exposures to Thiomersal-Containing Products

Thiomersal has been implicated in contact allergy (delayed-type hypersensitivity) skin reactions.[53,56,57] Between 1% to 16% of tested individuals exhibit allergic reactions on skin patch testing.[53]

Immediate hypersensitivity (e.g. anaphylaxis) and immune complex-mediated disorders (e.g. glomerulonephritis) have also been reported in association with exposure to thiomersal-containing products, although it is uncertain if thiomersal was the responsible allergen.[14,29]

3.4 Changes in Blood Mercury after use of Thiomersal-Containing Vaccines in Infants

Two studies have examined changes in blood mercury following immunisation with thiomersal-containing vaccines.[11,58] Pichichero et al.[11] compared the blood mercury levels of vaccine-derived thiomersal-exposed and control infants who were 2 months and 6 months of age. The cumulative mean vaccine-derived ethylmercury exposures were 45.6µg and 111.3µg for the 2- and 6-month-old infants, respectively. Twelve of 17 exposed 2-month-old infants had detectable blood mercury at a mean concentration of 8.2 nmol/L (or 1.6 µg/L), [SD = 4.9 nmol/L], whereas for 6-month-old exposed infants, 9 of 16 had detectable blood mercury at a mean concentration of 5.2 nmol/L (or 1.0 µg/L), [SD = 1.2 nmol/L]. By comparison, only one of eight 2-month-old control infants had detectable blood mercury of 4.65 nmol/L (or 0.9 µg/L) and none of seven 6-month-old control infants had detectable blood mercury. Prenatal mercury exposure, which was assessed by maternal hair analysis, did not differ, with mean mercury concentrations of 0.45 µg/g and 0.32 µg/g for mothers of vaccinated and control infants respectively (p = 0.22).

Stajich et al.[58] reported that immunisation with a single dose of hepatitis B vaccine containing 12.5µg of ethylmercury resulted in an increase in mean mercury blood level in pre-term infants from a baseline level of 0.54 µg/L (SD = ±0.79) to 7.36 µg/L (SD = ± 4.99), whereas in term infants an increase from a baseline of 0.04 µg/L (SD = ±0.09) to 2.24 µg/L (SD = ± 0.58) was detected, when measured 2–3 days after vaccination. Although pre-term infants received higher µg/kg doses than term infants, the ratio of prevaccination blood mercury concentration between pre-term and term infants was greater than the corresponding ratio of post-vaccination blood mercury concentration. This indicates that pre-term infants excreted a greater proportion of the mercury dose per kg bodyweight than term infants.[46,47]

It remains uncertain whether the higher levels of blood mercury detected in low-weight or pre-term infants following immunisation with thiomersal-containing vaccines pose any measurable toxicological risk.[47]

3.5 Evidence of Dose-Response Relationship from High-Dose Exposures to Ethylmercury

A report of short-term, high-dose exposure to ethylmercury in Iraq documented tremor with or without paraesthesia in three individuals with blood mercury concentrations of 1000, 1500, and 1700 µg/L, while no adverse effect was observed in an exposed fourth individual, who had a blood mercury level of 650 µg/L.[59] In China during the early 1980s, consumption of ethylmercury-treated rice caused a range of neurological symptoms including weakness, dizziness, numbness, paraesthesia and ataxia that were recognised in the mildest-affected persons at total dose exposure levels of 0.5–1 mg/kg bodyweight.[60] Magos[44] reported that no adverse effect was observed at blood mercury levels between 140 and 650 µg/L in five adults, assessed 11–22 days after exposure to varying doses of ethylmercury from contaminated food, infusion or topical application of ethylmercury-containing therapeutic/pharmaceutical products.[42] In this same review, the lowest observable adverse effect level was at a blood mercury level of 1000 µg/L.[42]

The lowest blood levels associated with adverse effects are approximately 1000 times higher than levels measured in 2-month-old infants following exposures reported by Pichichero et al.[11] Although dose-response relationships have been constructed for prenatal exposures to methylmercury,[61-64] no relationship between dose and response has been established for postnatal exposures to ethylmercury at the doses delivered through vaccines.[27]

4. Mercury Exposure Guidelines: Their Limitations and Assumptions

There have been no studies specifically designed to evaluate a ‘no observed effect level’ for ethylmercury,[1] although Magos[44] has interpolated data from published case reports. Although no ‘tolerable

daily intake' level for ethylmercury has been proposed,[65] various agencies have published recommended mercury exposure limits that provide policy-making guidance in managing long-term population exposure.[40-43,66-68] In general, these limits are intended to be protective of the fetus.[3,5,14,69] These exposure limits are back-calculated from hair or blood concentrations that are at a steady state and are intended for application to long-term, average daily intake of methylmercury from all sources (Health Canada, WHO, US Environmental Protection Agency and US FDA) or for *a minimum* of 1 year (Agency for Toxic Substances and Disease Registry), below which there is no known, appreciable health risk.[8,29,70] The exposure limits do not represent absolute levels above which toxicity occurs.[1,15,27,29,70] As these recommendations are intended to apply to average long-term, rather than a maximum single-day exposure, care must be taken to compare exposures averaged over a suitable time base. It is not meaningful to compare these guidelines with single-day exposures, such as the day on which a patient receives one or more thiomersal-containing vaccines.

To illustrate how these exposure guidelines can be misinterpreted by using inappropriate averaging times,[71] hypothetical 'worst-case' scenarios of calculated cumulative exposure limit to methylmercury exposure are depicted in figure 1 for infants at 14 weeks of age (corresponding with completion of EPI-recommended vaccinations at 14 weeks age), 6 months of age (approximating completion of similar primary vaccinations in developed countries) and at 1 year of age. These scenarios assume that a newborn female infant in the lowest fifth percentile of mean bodyweight receives all vaccines according to

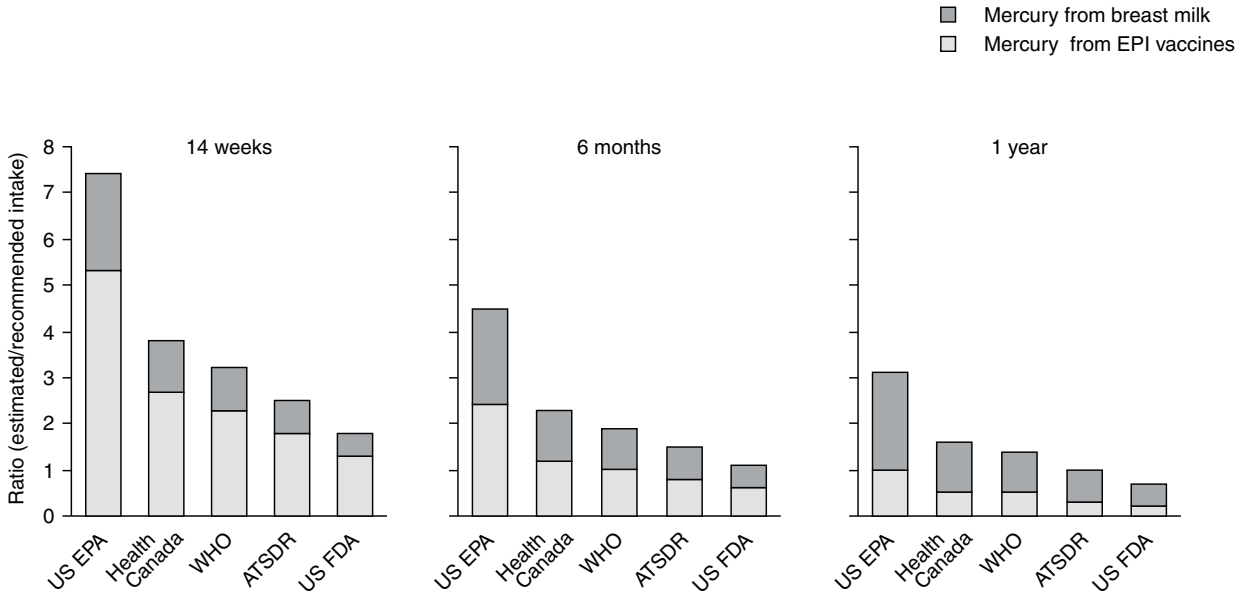

Fig. 1. Ratios of average weekly exposure to mercury from thiomersal-containing vaccines and/or breast milk, compared with agencies' exposure recommendations for methylmercury averaged over 14 weeks, 6 months and 1 year – hypothetical 'worst-case scenarios' for infants aged 14 weeks, 6 months and 1 year and based on fifth percentile of female infant bodyweight. Thiomersal-containing vaccine-derived mercury exposure was based on a cumulative ethylmercury dose of 187.5μg following immunisation according to WHO/EPI schedule up to 14 weeks of age. Breast milk-derived mercury exposure was calculated as: mean weight × mean daily breast milk consumption (140 mL/kg) × number of days × mean mercury concentration in breast milk (1.5 μg/L); mean weight was calculated using fifth percentile of weight for a female infant (birth weight 2.6kg) for specified ages (4.5kg at 14 weeks, 5.9kg at 6 months and 8.0kg at 1 year of age), i.e. mean weights of 3.6kg at 14 weeks, 4.3kg at 6 months and 5.3kg at 1 year of age.[72] The agencies' methylmercury exposure limits were calculated as dosage/kg bodyweight/week × mean weight × age in weeks (i.e. at a dosage of μg Hg/kg/week): WHO – 1.6;[40] US EPA – 0.7;[41] US FDA – 2.8 (estimate derived from an acceptable daily intake of 30 μg /day) [29,37]; ATSDR – 2.1;[43] Health Canada – 1.4.[66] Percentiles for female weight-by-age from US National Center for Health Statistics, published 30 May 2000 (modified 20 April 2001).[72] **ATSDR** = Agency for Toxic Substances and Disease Registry; **US EPA** = US Environmental Protection Agency; **WHO/EPI** = WHO Expanded Programme on Immunisation.

the WHO/EPI recommended schedule (table II), which corresponds to a cumulative dose of ethylmercury up to 187.5µg. The ratio of cumulative vaccine-derived ethylmercury exposure to agencies' calculated cumulative exposure limits for methylmercury rapidly decreases with age.

Since the exposure limit guidelines are derived from similar scientific data, the differences between agencies reflect varying assumptions and uncertainty factors that are applied in translating scientific data into public policy recommendations. None of the recommended exposure guidelines for mercury incorporates any consideration of offsetting health benefits that would be lost as a result of avoiding certain recognised sources of mercury exposure (e.g. protection against vaccine-preventable disease or reduction in cardiovascular disease risk from eating fish).[13] This reflects the guidelines' intended purpose to minimise *mercury-related* risks to health, not *overall* risks to health. For this reason, sound decision making about reducing health risks to individuals or populations must include all relevant risk information and not rely solely on exposure guidelines that consider only part of the total risk.

5. Other Sources of Mercury Exposure in Infants

Typical dietary consumption of some fish species by pregnant or lactating women, can result in fetal or infant mercury exposure approximating those from thiomersal-containing vaccines.[14,54,69,73-77] Recent estimates of breastfed infants' dietary mercury exposure from breast milk under normal environmental conditions range from <1 µg/L to approximately 3 µg/L.[11,76,78-80] A mean mercury concentration in breast milk of 1.5 µg/L, consumed by an exclusively breastfed,[81,82] fifth percentile female infant (mean bodyweight 4.3 kg), with an average intake of 140 ml/kg bodyweight per day of breast milk,[83] corresponds to a cumulative exposure to 164µg dietary mercury during the first 6 months of life. Thus, an exclusively breastfed infant is potentially exposed to approximately the same cumulative amount of mercury from breast milk in the first 6 months of postnatal life as from all WHO/EPI-recommended childhood vaccinations. Given that the same mercury exposure from vaccines occurs as three or four parenteral boluses, one could expect short-term, peak levels of mercury after vaccination to be higher than from ingesting breast milk. However, in either case no measurable adverse health effect from mercury has been recognised at this level of cumulative dose exposure during infancy.

6. Comparative Cumulative Mean Lifetime Mercury Exposures

Table IV places the relative exposures from vaccines in context with other common sources of exposure. Based on daily mercury exposures depicted, exposure to an average 2.4µg of methylmercury per day from fish consumption over an average lifetime of 65 years would result in a lifetime exposure of 57mg. This exceeds the lifetime vaccine-derived ethylmercury exposure from current recommended WHO/EPI childhood and adult immunisations (312.5µg) by a factor of 182. These comparisons do not mean that we should not try to reduce mercury exposure where possible but they do make clear that a <1% reduction in overall lifetime organic mercury exposure can be achieved by eliminating thiomersal from vaccines.

Table IV. Estimated daily intake/retention of elemental and mercuric compounds in a general population not occupationally exposed to mercury[84]

Exposure	Elemental mercury vapour (µg/day)		Inorganic mercury compounds (µg/day)		Methylmercury (µg/day)	
	intake	retention	intake	retention	intake	retention
Air	0.030	0.024	0.002	0.001	0.008	0.0064
Food – fish	0	0	0.600	0.042	2.4	2.3
Food – non-fish	0	0	3.6	0.25	0	0
Drinking water	0	0	0.050	0.0035	0	0
Dental amalgam	3.8–21	3–17	0	0	0	0
Total	**3.9–21**	**3.1–17**	**4.3**	**0.3**	**2.41**	**2.31**

7. Global Burden of Diseases for Which Thiomersal-Containing Vaccines are Available

In 2002, an estimated 500 000 children died of vaccine-preventable pertussis or tetanus,[85] while in 2000, an estimated 37 million children worldwide did not receive the routine immunisations in the first year of life that are recommended by WHO/EPI.[32] Immunisation coverage among infants for three doses of diphtheria-tetanus-pertussis vaccine was only 60% in Africa and approximately 70% in South-East Asia.[86] In 2003, over two dozen countries worldwide, mostly African nations designated as high endemic areas for chronic hepatitis B virus (HBV) infection [i.e. >8% prevalence],[87] had still not introduced HBV vaccine into their national infant immunisation programmes.[88] Among many developing countries that report having implemented HBV immunisation programmes, immunisation coverage is seriously compromised by healthcare system financial constraints.[32,89]

8. Implications of Changing Thiomersal Content on Vaccine Effectiveness and Safety

Although thiomersal is added to vaccines primarily as a preservative, it has also been shown to improve vaccine stability, potency and safety.[26,28] In some production processes, such as the manufacture of whole-cell pertussis vaccine, thiomersal is used in conjunction with heat to inactivate bacterial antigen.[25,90] Thimerosal may also be added to some formulations of bulk vaccine prior to filling into final containers as a substitute to filtration-sterilisation.[90] Traces of organic mercury may also have a stabilising effect on vaccine antigens, such as the semi-synthetically produced HBV surface antigen in recombinant HBV vaccines and in whole-cell pertussis vaccine.[90] Its reduction, elimination or replacement from certain vaccines could therefore adversely affect vaccine quality, safety and efficacy. Extensive characterisation, pre-clinical and clinical testing of replacement products will likely be necessary prior to licensure by regulatory authorities.[25,28,90,91] An extended 28 day shelf life has also been approved by WHO following initial use of thiomersal-containing, multi-dose vaccine vials.[92] This extended shelf life does not necessarily apply for alternative vaccine preservatives such as 2-phenoxyethanol or formaldehyde, which are not as effective as thiomersal in terms of bacteriostatic properties.[91]

9. Cost Implications of Changing Thiomersal Content in Vaccines

A significant concern of WHO is the negative impact of thiomersal removal on vaccine production capacity and cost to developing countries. In 2001, 48 countries (including many in the developing world) had domestic vaccine production facilities.[32] As much as 60% of vaccine production in the developing world is used domestically, mostly manufactured as multi-dose, thiomersal-containing vaccines.[1] Multi-dose vials appear to be most appropriate for less expensive (e.g. WHO/EPI-recommended) vaccines and where cold chain systems are very limited.[93]

Despite economic evaluations indicating that childhood immunisation is highly desirable in developing countries,[94] vaccine population coverage can be highly sensitive to even small price increments.[95] In developing countries, which are the primary focus of the WHO/EPI immunisation programme, the total vaccine programme delivery cost per child for complete immunisation with bacilli Calmette-Guérin, diphtheria-tetanus-pertussis, polio and measles vaccines is about $US17,[96] of which vaccine cost represents probably <$US1.[97] Although vaccine cost of $US1 per child may not seem significant, this should be viewed in the context of total government expenditures on health of <$US10 per capita per year in many developing countries.[85] Use of single dose thiomersal-free vaccine formats could raise the overall expense of vaccination programmes and jeopardise the cost effectiveness of immunisation programmes in developing world settings by increasing infrastructure costs related to storage space, containers, container filling, transportation and maintaining adequate cold chain.[91]

10. Different Strategies for Managing Potential Risks and Benefits of Thiomersal-Containing Vaccines

From the perspective of individual risk, the absence of credible evidence linking thiomersal-containing vaccines to mercury-related effects on health and the demonstrated reduction in risk of vaccine-preventable disease indicate that the 'low risk' choice is to immunise with thiomersal-containing vaccines rather than not to immunise because of fears of mercury-related effects on health. That said, in settings such as more affluent countries, it may be perfectly rational to 'prefer' thiomersal-free vaccines to thiomersal-containing vaccines, both on precautionary grounds and to reduce overall exposure to mercury, particularly during infancy.

However, even in affluent countries, individual choice and access to alternative vaccine products may be constrained by pharmaceutical industry product marketing or government vaccine procurement policies for publicly funded immunisation programmes. Consumer preference can be a powerful force for affecting change and much of the impetus for improvements in consumer products and pharmaceuticals in many countries is driven by consumer demand, both directly and through actions of government agencies.

From the perspective of population-based risk, choices are more complicated when it comes to developing national or regional policies on thiomersal-containing vaccines. By-and-large, affluent countries have opted to move towards thiomersal-free, single-dose formats of vaccine for immunisations routinely recommended for children. This decision reflects a desire to maintain protection against vaccine-preventable disease while avoiding or reducing overall exposure to mercury, regardless of any established proven benefit from such exposure reductions. This choice of vaccine products in affluent countries is made possible by a willingness to absorb higher total vaccine programme costs and by having sufficient vaccine production capacity to transition relatively quickly to thiomersal-free products.[1,32] It also reflects a growing concern among parents, fueled by vocal, activist, anti-vaccination groups, about the safety of vaccines.[98] Failure by public policy makers and health officials to respond to these concerns could result in reduced vaccine uptake in the population and a net increase in the risk of vaccine-preventable disease at a population level.

On the other hand, in developing countries, choice is often more limited and the stakes are higher. As the rates of many vaccine-preventable diseases are higher, the benefits of immunisation are greater, as are the risks of failing to immunise or even deferring immunisation. Unlike more affluent countries, there are significant limitations in healthcare resources and vaccine storage, handling and delivery infrastructure. In this setting, immunisation programmes using current thiomersal-containing, multi-dose vaccines are one of the most highly cost-effective – even cost-saving – health strategies. In developing countries, as in more affluent countries, the 'highest risk' option is the failure to immunise.

In light of the relatively greater cost implications and practical difficulties in delivering vaccine programmes in developing countries, thiomersal should probably only be replaced in these countries when suitable safe, effective alternatives that produce equivalent or lower costs for total vaccine programme delivery become available.[28] Over time, thiomersal-free vaccines can be systematically introduced to replace low-cost, multi-dose thiomersal-containing combination vaccines that, when administered according to WHO/EPI recommendations, have proven to be so effective in protecting children.

11. Conclusions

The health risks from vaccine-preventable diseases are well documented and are generally far higher in developing countries than in affluent countries. While the toxicity of mercury at high doses is well established, the risks from low-level exposure to thiomersal-containing vaccines are speculative and inadequately quantified.

Removal of thiomersal from vaccines will reduce exposure to mercury, particularly during infancy. Regulatory requirements mandating mercury exposure reduction, along with concern about potential risks to health, has led to the deployment of thiomersal-free vaccines in many countries that are able to afford higher-priced vaccines. Ideally, immunisa-

tion against vaccine-preventable diseases should be provided without incurring mercury exposure. However, the risks of failing to immunise against vaccine-preventable disease outweigh the possible risk associated with mercury in vaccines, particularly in developing nations. For this reason, thiomersal-containing vaccines are a 'safer' choice than no vaccines at all and these vaccines should continue to be employed, especially in developing countries, until thiomersal-free substitutes become a practical, affordable alternative.

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The US EPA reference dose for methylmercury: sources of uncertainty ☆

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Abstract

The US Environmental Protection Agency (EPA) derived a reference dose for methylmercury in 2001, based on an extensive analysis by the National Research Council (NRC) of the National Academy of Sciences. The NRC performed benchmark dose analysis on a number of endpoints from three longitudinal prospective studies: the Seychelles Islands, the Faroe Islands, and the New Zealand studies. Adverse effects were reported in the latter two studies, but not in the Seychelles study. The NRC also performed an integrative analysis of all three studies. Dose conversion from cord blood or maternal hair mercury concentration was performed by EPA using a one-compartment pharmacokinetic model. A total uncertainty factor of 10 was applied for intrahuman pharmacokinetic and pharmacodynamic variability. There are numerous decisions made by the NRC/EPA that could greatly affect the value of the reference dose (RfD). Some of these include the choice of a linear model for the relationship between mercury body burden and neuropsychological performance, the choice of values of P_0 and the benchmark response, the use of the "critical study/critical endpoint" approach in the interpretation of the maternal body burden that corresponds to the RfD, the use of central tendencies in a one-compartment pharmacokinetic model rather than the inclusion of the distributions of variables for the population of reproductive-age women, the assumption of unity for the ratio of fetal cord blood to maternal blood methylmercury concentrations, the choice of a total of 10 as an uncertainty factor, and the lack of dose–response analysis for other health effects such as cardiovascular disease. In addition, it may be argued that derivation of a RfD for methylmercury is inappropriate, given that there does not appear to be a threshold for adverse neuropsychological effects based on available data.

1. Introduction

The US Environmental Protection Agency (EPA) has, as part of its mandate, a responsibility to perform risk assessments for chemicals present in the environment that may pose a hazard to human health. As part of risk assessment for noncancer effects, the EPA may derive a reference dose (RfD), defined as "an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime." The EPA derived an RfD for methylmercury in 2001, based on an analysis of the health effects of methylmercury by the National Research Council (NRC) (NRC, 2000). The summary of the EPA derivation is available on the EPA website (URL: http://www.epa.gov/iris/subst/0073.htm) as well as in the published literature (Rice et al., 2003). The background document from which these summaries are extracted is also available (US EPA, 2001; URL: http://www.epa.gov/waterscience/criteria/methylmercury/criteria.html). This paper will briefly summarize the decisions made by the EPA in the derivation of the RfD, some areas of variability and uncertainty not addressed in the assessment, and additional analyses that could be

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performed using existing data and that would provide important information useful for the risk assessment of methylmercury.

2. Summary of the NRC/EPA analysis

The NRC based its evaluation on three epidemiological studies suitable for quantitative analysis. These longitudinal prospective developmental studies were conducted in the Seychelles Islands, the Faroe Islands, and New Zealand. The Seychelles Islands study consisted of 779 mother–infant pairs from a fish-eating population (Davidson et al., 1995, 1998; Myers et al., 1995a–c, 1997). Infants were followed from birth to 5.5 years of age and assessed at various ages on a number of standardized neuropsychological endpoints. The independent variable was maternal hair mercury concentrations. The Faroe Islands study included about 900 mother–infant pairs; children were tested on a variety of tasks at 7 years of age (Grandjean et al., 1997). The main independent variable was cord blood mercury, although maternal hair mercury was also measured. In the New Zealand study, 38 children of mothers with hair mercury levels during pregnancy greater than 6 ppm were matched with children whose mothers had had lower hair mercury concentrations (Kjellstrom et al., 1986, 1989). At 6 years of age, a total of 237 children were assessed on a number of neuropsychological endpoints similar to those used in the Seychelles study (Kjellstrom et al., 1989). Investigators in the Seychelles Islands study reported no evidence of impairment related to in utero methylmercury exposure in their main study, whereas the other two studies found exposure-related effects on a number of neuropsychological endpoints.

The NRC performed bench mark dose (BMD) analysis on five endpoints from the Faroe Islands study from a total of nine that had been reported as significantly affected by methylmercury exposure (Grandjean et al., 1997) (Table 1). Similarly, five endpoints negatively associated with methylmercury exposure in the New Zealand study (Kjellstrom et al., 1989) were used in the BMD analysis by the NRC. All of the endpoints assessed in the Seychelles study were also modeled, even though the Seychelles study was reported as negative. In BMD analysis, the first step is to model the relationship between the endpoint (neuropsychological performance) and exposure (body burden). The NRC used a linear model for all analyses. BMD analysis

Table 1
Tests modeled by NRC, functions assessed, and potential societal relevance

Study	Test	Domain/function assessed	Societal relevance
Seychelles	Bender copying errors	Visuospatial	Math performance
	McCarthy GCI	Full-scale IQ	School performance, intelligence
	WJ applied problems	Ability to solve problems	Academic skills
	CBCL	Social and adaptive behavior	Antisocial behavior, need for therapeutic services
	Preschool language scale	Broad-based language	Learning, intelligence, school performance
	WJ letter/word recognition	Word recognition	Reading ability, school performance
Faroes	Finger tapping	Motor performance	Motor speed/neuropathy
	CPT reaction time	Vigilance, attention, information and processing speed	Intelligence, school behavior, performance
	Bender copying errors	Visuospatial	Math performance
	Boston naming test	Expressive vocabulary	Reading, school performance
	CVLT: delayed recall	Memory	Learning ability, school performance
New Zealand	TOLD language development	Broad-based language	Literacy skills, learning, school performance
	WISC-R: PIQ	Performance IQ, e.g., visuospatial, sustained attention, sequential memory	Learning, school performance
	WISC-R: FSIQ	Full-scale IQ, e.g., PIQ + verbal processing, expressive vocabulary	Learning, school performance
	McCarthy perceptual performance	Performance IQ, e.g., visuospatial, audition, memory	Learning, school performance
	McCarthy motor test	Gross and fine motor skills	Motor system integration

Abbreviations used: GCI, General Cognitive Index; WJ, Woodcock–Johnson tests of achievement; CBCL, child behavior check list; CPT, continuous performance test; CVLT, California verbal learning test; TOLD, test of language development; WISC-R:PIQ, Wechsler intelligence scale for children–revised performance IQ; WISC-R:FSIQ, Wechsler intelligence scale for children–revised full-scale IQ.
From US EPA (2001, pp. 4–51).

requires two additional decisions once an appropriate model has been chosen. When continuous data are used, a point on the curve below which responses are considered "abnormal" must be chosen, termed P_0. A value of $P_0 = 0.05$ was used in the NRC/EPA assessment: that is, the cut-off for abnormal response was set at the lowest 5% (5th percentile) of children. The second decision that must be made is the choice of the increase in the proportion of individuals that will be expected to perform in the abnormal category in an exposed versus an unexposed population. This is defined as the bench mark response (BMR). A BMR of 0.05 was chosen for this assessment, which would result in a doubling of the number of children with a response at or below the 5th percentile in an unexposed population.

BMDs were calculated for each of the endpoints described above for each of the three studies. The lower limit on the 95% confidence interval of the BMD (the BMDL) was calculated for each endpoint (Table 2). The BMDLs from the Faroe Islands study were 12–15 ppm total mercury in maternal hair, whereas those in the New Zealand study were 4–6 ppm. The BMDLs from the Seychelles Islands study were 17–25, about 50% higher than those in the Faroe Islands and 250–300% higher than those from the New Zealand study. It is important to recognize that the BMDL represents a defined risk level: in this case, a doubling of the number of children performing in the abnormal range. It is therefore not equivalent to the NOAEL (no observed adverse effect level), which by definition is a level at which no adverse effects are identified. These BMDLs served as potential points of departure (PODs) for the RfD. The POD is the starting point in the risk assessment to which uncertainty factors (UFs) are applied, in order to ensure that the resultant RfD is sufficiently health protective. The EPA applied an uncertainty factor of 10 to each POD to account for intrahuman variability: that is, each POD was divided by 10.

Table 2
BMDLs, ingested dose, and RfDs for various endpoints from the Faroes Islands, New Zealand, and the NRC integrative analysis[a]

Test[b]	BMDL ppb mercury cord blood	Ingested dose (μg/kg/day)[c]	RfD (μg/kg/day)[d]
BNT Faroes			
Whole cohort	58	1.081	0.1
PCB adjusted	71	1.323	0.1
Lowest PCB	40	0.745	0.1
CPT Faroes			
Whole cohort	46	0.857	0.1
PCB adjusted	49	0.913	0.1
Lowest PCB	28	0.522	0.05
CVLT Faroes			
Whole cohort	103	1.920	0.2
PCB adjusted	78	1.454	0.1
Lowest PCB	52	0.969	0.1
Finger tap faroes			
Whole cohort	79	1.472	0.1
PCB adjusted	66	1.230	0.1
Lowest PCB	24	0.447	0.05
Geometric mean Faroes			
Whole cohort	68	1.268	0.1
PCB adjusted	65	1.212	0.1
Lowest PCB	34	0.634	0.1
Smoothed values			
BNT Faroes	48	0.895	0.1
CPT Faroes	48	0.895	0.1
CVLT Faroes	60	1.118	0.1
Finger tap Faroes	52	0.969	0.1
MCCPP New Zealand	28	0.522	0.05
MCMT New Zealand	32	0.596	0.1
Median values			
Faroes	48	0.895	0.1
New Zealand	24	0.447	0.05
Integrative			
All endpoints	32	0.596	0.1

From US EPA (2001, pp. 4–61).

[a] $BMDL_{05}$'s from NRC (2000), Tables 7-4, 7-5, 7-6. Total hair mercury was converted to blood mercury for the New Zealand and Seychelles Islands studies using a 250:1 ratio and an assumption of equivalent maternal and cord levels.

[b] Abbreviations used: BNT, Boston naming test; CPT, continuous performance test; CVLT, California verbal learning test; MCCPP, McCarthy perceived performance; MCMT, McCarthy motor test.

[c] Calculated using a one-compartment model.

[d] Calculated using an UF of 10.

3. Use of the linear model for the relationship between body burden and adverse outcome

The NRC modeled the dose–effect relationship for the endpoints from the three studies identified as suitable for quantitative analysis using the K power model and determined the K value that best fit the data. The NRC constrained the model to $K \geqslant 1$. This allowed a sublinear relationship: i.e., a lower slope at lower body burdens and a comparatively greater slope at higher body burdens. The NRC reasoned that a supralinear model was biologically implausible. Under these conditions, the best fit to the data was $K = 1$, or a linear dose–effect relationship, which was the model used for all endpoints from all three studies. In fact, for the Faroe Islands endpoints, supralinear models such as the square root or logarithmic transformations were a better fit than the linear model (Budtz-Jørgensen et al., 1999, 2000). In other words, there was evidence that the slope was

actually steeper at lower body burdens than at higher ones. This was also the case for the endpoints from the New Zealand study (Louise Ryan, statistician on the NRC panel, personal communication). A disadvantage of the alternative models, at least for the Faroe Islands study, was a larger difference in the BMDs between endpoints compared to the linear model, as well as instability in the 95% confidence interval (which yields the BMDL). However, it may in fact be plausible that the dose–effect function is steeper at lower doses, before exposures are reached at which compensatory mechanisms are activated. There is evidence that the effects of lead on cognitive function in children may be supralinear, for example (Canfield et al., 2003; Bellinger and Needleman, 2003; Fulton et al., 1987; Lanphear et al., 2000; Schwartz, 1994). The decision to use a linear model had a large effect on the calculation of the BMDLs, which would have been considerably lower if, for example, a log-linear model had been used. This is an area that requires further exploration not only in terms of dose–effect modeling, but also with regard to basic neurochemical and neurophysical mechanisms that may underlie the observed behavioral effects.

4. Choice of P_0 and BMR

The values of both P_0 and the BMR have significant impact on the POD. A choice of a higher BMR, for example from a doubling of an abnormal response to a tripling, obviously would result in a higher POD. Conversely, the choice of a lower P_0 would result in a higher POD, and a higher P_0 would result in a lower POD. The NRC and EPA chose the fifth percentile, which would be roughly comparable to an IQ of 75 in terms of population distribution. The first percentile corresponds to an IQ of 65, in the range of clinical mental retardation. The 10th percentile corresponds with an IQ of 81. An IQ of neither 75 nor 81 is considered to lie within the clinical definition of mental retardation. However, individuals with neuropsychological functioning within this range will have difficulty functioning in our highly technological society and may require special educational and other services. The NRC apparently performed no analyses of the differential costs to society of individuals at various low functional levels as a basis for the choice of the fifth percentile, nor did the EPA address the issue. Such analyses could inform the choice of both P_0 and the BMR.

5. Use of the "critical study/critical endpoint" approach

In past assessments the EPA typically derived RfDs based on the choice of a single study (the "critical study") and a single endpoint from that study (the "critical endpoint"). However, it is not EPA policy that assessments be performed using that strategy, and a recent review of the RfD/RfC derivation process recommends using more of the available data in determining reference values (US EPA, 2003). In its deliberations, the NRC identified a critical study (the Faroe Islands study) and a critical endpoint [Boston naming test (BNT) on the full cohort] in order to conform to standard practices. However, the panel also performed an integrative analysis of all three studies in order to encourage that direction in risk assessment (David Bellinger, NRC panel member, personal communication).

In the document written by the EPA for the derivation of the methylmercury RfD (US EPA, 2001), the BNT from the Faroe Islands study was used as an example for the purpose of illustrating the calculations for the dose conversion using the one-compartment model. The BMDL for the BNT is 58 ppb mercury in cord blood (Table 2). This number has been used both inside and outside the EPA to represent the body burden that corresponds to the RfD; e.g., to estimate the fraction of US women considered to have blood mercury concentrations that exceed the RfD (Schober et al., 2003). However, the EPA considered the RfD to be based on the totality of evidence from the Faroe Islands and New Zealand studies, as well as the integrative analysis of all three studies, including the negative Seychelles Islands study.[1] Numerous other endpoints also yielded an RfD of 0.1 μg/kg/day. For example, the geometric mean for the full cohort of the Faroe Islands study from the endpoints modeled by the NRC is 68 ppb, whereas the median value from that study is 48 ppb. The smoothed value for the BNT, in which the effect of high and low points is attenuated, is also 48 ppb. The integrative analysis of all the data resulted in a maternal blood concentration of 32 ppb. This last number may be considered a more reliable indicator of all of the available data than the critical study/critical endpoint approach. In any case, the choice of blood mercury concentration that is deemed to represent the RfD has not been adequately evaluated and should be revisited in light of the recent review of the RfD/RfC process and the fact that the BNT is being interpreted as the body burden that represents the RfD.

6. Use of the one-compartment model for conversion of body burden to maternal intake

The neurotoxic effects associated with methylmercury exposure were modeled based on cord blood and/or

[1] "Rather than choose a single measure for the RfD critical endpoint, EPA based the RfD for this assessment on several scores from the Faroes measures, with supporting analyses from the New Zealand study, and the integrative analysis of all three studies." Iris summary for methylmercury, http://www.epa.gov/iris/subst/0073.htm, p. 7.

maternal hair mercury concentrations. To derive an RfD, a daily intake of methylmercury that results in the modeled PODs must be estimated. The EPA used a one-compartment pharmacokinetic model to convert cord blood (or maternal hair) to maternal intake,

$$d = \frac{C \times b \times V}{A \times f \times \mathrm{bw}},$$

where C = (BMDL), b = maternal elimination constant (0.014/days), V = maternal blood volume (5 L), A = fraction of absorbed intake (0.95), f = fraction of absorbed dose in blood (0.059), and bw = maternal body weight (67 kg). There are numerous sources of uncertainty associated with this conversion.

A one-compartment model is a significant simplification of the pharmacokinetics of methylmercury in the maternal body and maternal–fetal unit. Physiological-based pharmacokinetic models have been developed for methylmercury (Swartout and Rice, 2000). However, accurate rate constants are not available for humans for the necessary compartments, including maternal blood $\rightleftharpoons$ fetal blood $\rightleftharpoons$ fetus and fetal organs. The one-compartment model predicts the elimination half-life from maternal blood reasonably well (Ginsberg and Toal, 2000) and thus is useful for estimating maternal blood half-life.

7. Use of central tendency estimates for equation parameters

The choice of the values for each parameter of the equation was based on a thorough review of available data. Adequate data were available for a central tendency estimate for some parameters, such as the body weight of American women and the elimination half-life, but not for others, such as blood volume in American women at the end of pregnancy. Perhaps more important, however, is the fact that this approach does not address the variance in these parameters and thereby the variance in the relationship between methylmercury intake and body burden. For example, elimination half-lives in humans may vary from 30 days to as long as 120 days (WHO, 1990; Al-Shahristani and Shihab, 1974). Similarly, there are large differences in the body weights of US women. The distributions of these variables are not captured in the model. It is unknown whether the current UF of 10 is sufficient to include the compound variability of these factors.

8. Ratio of cord blood: maternal blood methylmercury concentrations

The EPA assumed that the ratio of methylmercury in fetal cord blood compared to maternal blood was 1:1 for the conversion of cord blood to maternal intake. The EPA identified this as an area of uncertainty that required further investigation. A Monte Carlo analysis based on 10 published studies that met inclusion criteria estimated the ratio of cord blood:maternal blood at 1.6–1.8:1, with the 95th percentile being over 3.0 (Stern and Smith, 2003). The greater concentration of methylmercury in cord blood compared to maternal blood, based on central tendency, would result in a concomitant decrease in the RfD, assuming other decisions were not changed. In addition, the variance in the ratio needs to be included in the estimation of intrahuman variability.

9. Conversion of maternal hair mercury to maternal blood methylmercury

Cord blood mercury concentrations were only available for the Faroe Islands study. For the New Zealand and Seychelles Islands studies, only maternal hair mercury concentrations were available. In its analyses, the NRC assumed a ratio of 250:1 to convert from maternal hair to maternal blood, with no estimate of variance. The maternal hair concentrations are not directly related to the dose to the fetus, as are cord blood concentrations. Nonetheless, accurate estimates of central tendency and the variance are important for dose conversion. Preliminary weighted analyses of NHANES 99+ suggest that the central tendency is different from 250, with large variation between individuals. This database should be evaluated further with respect to derivation of the BMDs for the New Zealand and Seychelles studies.

10. Choice of uncertainty factor

The EPA used a total uncertainty factor of 10 from each POD to calculate interim RfDs from the endpoints modeled by the NAS. This was considered to include a factor of 3 for variability in maternal elimination half-life and a factor of 3 for pharmacodynamic variability. The former was based on analyses of the variability in human elimination half-life (Swartout and Rice, 2000; Stern, 1997; Clewell et al., 1999), whereas the latter was a default value. The UF also recognized the lack of quantification of cardiovascular effects and delayed neurotoxicity, as well as possible reproductive effects. As outlined above, the total pharmacokinetic variability for the dose conversion is most certainly greater than three. (Conversely, it could be argued that the pharmacodynamic variability is already included in the response. This assumes, however, that the small number of subjects in these studies are representative of the entire US population of women of childbearing potential.) In addition, the EPA did not apply a factor for the

fact that the PODs from the BMD analysis represent body burdens associated with a defined risk and in no way constitute a threshold or a NOAEL. In situations in which a low observable adverse effect level (LOAEL) but not a NOAEL is identified, the EPA default strategy is the application of an additional UF factor of 10. It can be argued that a POD from a BMD analysis is comparable to a LOAEL; it certainly is not a NOAEL. In the recent assessment of benzene, the EPA used a BMD analysis of lymphocyte counts in humans as the POD for setting a reference value. A factor of 3 was added to account for the fact that the POD was an effect level. In the case of methylmercury, the PODs were associated with adverse health effects rather than a precursor to an adverse effect, as was the case with benzene. The use of an additional UF for methylmercury clearly requires further evaluation.

11. Other endpoints not quantified in the current assessment

An endpoint identified by both the NRC and EPA as requiring further evaluation is cardiovascular toxicity associated with methylmercury intake from fish. A study of fish eaters in Finland reported an increase in carotid atherosclerosis, myocardial infarction, and death in men as a function of increased hair mercury (Salonen et al., 1995, 2000). A multicenter European study also reported a significant association between mercury body burden and the risk of myocardial infarction in men after controlling for levels of a fatty acid in fish thought to be cardio-protective (Guallar et al., 2002). Adverse effects were not identified based on total mercury levels in a population that included a large proportion of dentists, whose increased mercury body burdens probably represented occupational exposure (Yoshizawa et al., 2002). Separate anaylsis excluding the dentists revealed a trend toward adverse effects on cardivascular function that was nonsignificant. The EPA considers the RfD to be appropriate for everyone. However, a number of states develop two-tier fish advisories, with lower fish intakes recommended for women of childbearing potential and young children than for men and older women. This strategy is based on the assumption that the developing nervous system is the organ system most sensitive to the effects of methylmercury. This may not be true, however, since cardiovascular effects in men have been associated with hair mercury concentrations below 3 ppm.

There is evidence from both human and experimental studies that either developmental or adult exposure to moderate levels of methylmercury may result in delayed neurotoxicity years or decades after the cessation of exposure, often during aging (Rice, 1996; Kinjo et al., 1993). These effects include somatosensory impairment and impairment in other sensory systems in monkeys and sensorimotor impairment in humans sufficient to interfere with the ability to independently perform routine personal care. This consequence of methylmercury exposure has important societal implications, particularly considering the aging of the US population. However, it is unlikely that it will be possible to determine the exposure- or body burden-effect function for these effects.

12. Research to address uncertainties

The EPA has initiated research in a couple of areas relevant to a risk analysis of the human health effects of methylmercury.

- A BMD analysis of the cardiovascular effects of methylmercury in adult males is being performed. This analysis will allow a comparison between the neurodevelopmental toxicity that is the current basis for the EPA RfD and effects in adults.
- The recent Monte Carlo analysis by Stern and Smith (2003) will be integrated into a full distributional analysis of the one-compartment model for conversion of cord blood methylmercury concentration to maternal intake. Distributions will be generated for maternal elimination half-life, cord blood:maternal blood ratio, maternal body weight, maternal blood volume, and perhaps absorption constants. This analysis will allow an estimate of the distribution of maternal intakes that may result in any particular cord blood concentration, resulting in a much better estimate of the upper end of distribution of mother–fetal pairs at risk for elevated methylmercury exposure than was performed in the EPA's 2001 assessment.

13. The bigger picture

In the NRC analyses of the Faroe Islands and New Zealand studies, there was not evidence of a threshold for the effects of methylmercury within the range of the lowest body burdens in the study population, about 1–2 ppm in maternal hair. In fact, supralinear models actually provide a better fit to the data, as discussed above. Since derivation of an RfD assumes an identifiable threshold, this presents something of a dilemma. This situation is not unprecedented: e.g., the EPA has declined to derive an RfD for lead, reasoning that "[b]y comparison to most other environmental toxicants…the degree of uncertainty about the health effects of lead is quite low. It appears that some of these effects, particularly…aspects of children's neurobehavior development, may occur at blood lead levels so low as to be

essentially without a threshold'' (US EPA, 1991). The practical extension of that evaluation is that every effort be made to remove lead sources from the environments of children. The situation with methylmercury is more complicated, since the source of methylmercury exposure in the United States is virtually exclusively from fish, a good source of protein and health-protective fatty acids. This reality is currently addressed largely through state fish advisories, which attempt to balance the advantages of eating fish while protecting against potential methylmercury-induced adverse health effects. The long-term goal should be the decrease of new anthropogenic releases of methylmercury into the environment. In addition, however, the risk associated with methylmercury exposure needs to be further evaluated, whether that be through more accurate data for reevaluation of the RfD or new approaches with respect to risk assessment of methylmercury.

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EXHIBIT 370

United States Environmental Protection Agency

Methylmercury (MeHg)

CASRN 22967-92-6

- IRIS Summary (PDF) (44 pp, 258 K)
- Status: Methylmercury (MeHg) is in step 1 at this time; see Quick Check.

Key IRIS Values

Assessment Status

Chemical Documents

Other EPA Information

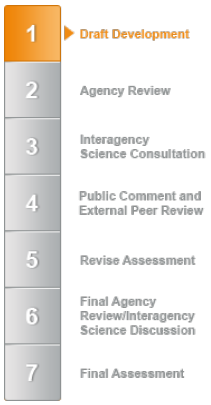
Noncancer Assessment

Reference Dose for Oral Exposure (RfD) (PDF) (44 pp, 258 K)

Last Updated: 07/27/2001

System	RfD (mg/kg-day)	Basis	PoD
Nervous, Developmental	1×10^{-4} (High end of BMDL05 range)	Developmental neuropsychological impairment	$BMDL_5$: 1.5×10^{-3} mg/kg-day
Nervous,	1×10^{-4} (Low end of BMDL05	Developmental neuropsychological	$BMDL_5$: 8.6×10^{-4}

Quick Check



Critical Effect Systems

Developmental

Developmental	range)	impairment	mg/kg-day

Nervous

Reference Concentration for Inhalation Exposure (RfC) (PDF) (44 pp, 258 K)
Not assessed under the IRIS Program.

Cancer Assessment

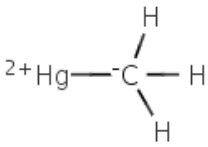
Weight of Evidence for Cancer (PDF) (44 pp, 258 K)
Last Updated: 05/01/1995

WOE Characterization	Framework for WOE Characterization
C (Possible human carcinogen)	Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986)

Basis:

- Based on inadequate data in humans and limited evidence of carcinogenicity in animals. Male ICR and B6C3F1 mice exposed to methylmercuric chloride in the diet had an increased incidence of renal adenomas, adenocarcinomas and carcinomas. The tumors were observed at a single site and in a single species and single sex. The renal epithelial cell hyperplasia and tumors were observed only in the presence of profound nephrotoxicity and were suggested to be a consequence of reparative changes in the cells. Several nonpositive cancer bioassays were also reported. Although genotoxicity test data suggest that methylmercury is capable of producing chromosomal and nuclear damage, there are also nonpositive genotoxicity data.
- This may be a synopsis of the full weight-of-evidence narrative.

Chemical Structure for Methylmercu (MeHg)



Synonyms

- MEHG
- Mercury (1+), methyl-, ion
- Mercury(1+), methyl-
- Methyl mercury
- Methylmercury

more synonyms

Quantitative Estimate of Carcinogenic Risk from Oral Exposure (PDF) (44 pp, 258 K)
Not assessed under the IRIS Program.

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure (PDF) (44 pp, 258 K)
Not assessed under the IRIS Program.

You will need Adobe Reader to view some of the files on this page. See EPA's PDF page to learn more.

Contact Us to ask a question, provide feedback or report a problem.

JULY 28, 2017

EXHIBIT 371

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Vaccine Safety

Thimerosal in Vaccines

Thimerosal is a mercury-based preservative that has been used for decades in the United States in multi-dose vials (vials containing more than one dose) of medicines and vaccines. There is no evidence of harm caused by the low doses of thimerosal in vaccines, except for minor reactions like redness and swelling at the injection site. However, in July 1999, the Public Health Service agencies, the American Academy of Pediatrics, and vaccine manufacturers agreed that thimerosal should be reduced or eliminated in vaccines as a precautionary measure.

Thimerosal contains ethylmercury.

Mercury is a naturally occurring element found in the earth's crust, air, soil, and water. Two types of mercury to which people may be exposed — methylmercury and ethylmercury — are very different.

Methylmercury is the type of mercury found in certain kinds of fish. At high exposure levels methylmercury can be toxic to people. In the United States, federal guidelines keep as much methylmercury as possible out of the environment and food, but over a lifetime, everyone is exposed to some methylmercury.

Thimerosal contains **ethylmercury**, which is cleared from the human body more quickly than methylmercury, and is therefore less likely to cause any harm.

Thimerosal prevents the growth of bacteria in vaccines.

Thimerosal is added to vials of vaccine that contain more than one dose (multi-dose vials) to prevent growth of germs, like bacteria and fungi. Introduction of bacteria and fungi has the potential to occur when a syringe needle enters a vial as a vaccine is being prepared for administration. Contamination by germs in a vaccine could cause severe local reactions, serious illness or death. In some vaccines, preservatives, including thimerosal, are added during the manufacturing process to prevent germ growth.

The human body eliminates thimerosal easily.

Thimerosal does not stay in the body a long time so it does not build up and reach harmful levels. When thimerosal enters the body, it breaks down to ethylmercury and thiosalicylate, which are readily eliminated.

Thimerosal has been shown to be safe when used in vaccines.

Thimerosal use in medical products has a record of being very safe. Data from many studies show no evidence of harm caused by the low doses of thimerosal in vaccines.

There are some side effects of thimerosal in vaccines.

The most common side-effects are minor reactions like redness and swelling at the injection site. Although rare, some people may be allergic to thimerosal.

Scientific research does not show a connection between thimerosal and autism.

Research does not show any link between thimerosal in vaccines and autism, a neurodevelopmental disorder. Many well conducted studies have concluded that thimerosal in vaccines does not contribute to the development of autism. Even after thimerosal was removed from almost all childhood vaccines, autism rates continued to increase, which is the opposite of what would be expected if thimerosal caused autism.

Thimerosal was taken out of childhood vaccines in the United States in 2001.

Measles, mumps, and rubella (MMR) vaccines do not and never did contain thimerosal. Varicella (chickenpox), inactivated polio (IPV), and pneumococcal conjugate vaccines have also never contained thimerosal.
Influenza (flu) vaccines are currently available in both thimerosal-containing (for multi-dose vaccine vials) and thimerosal-free versions.

For a complete list of vaccines and their thimerosal content level, see the U.S. Food and Drug Administration (FDA) Thimerosal in Vaccines page. This chart [PDF – 182 KB] shows vaccine ingredients sorted by vaccine.

Featured Resource: Understanding Thimerosal, Mercury, and Vaccine Safety [PDF – 300 KB]

Related Links

Frequently Asked Questions about Thimerosal

Ingredients of Vaccines – Fact Sheet

Vaccine Ingredients

CDC Studies on Vaccines and Autism [357 KB]

Thimerosal: What You Should Know (Spanish) [PDF - 2 pages]

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Content source: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare Quality Promotion (DHQP)

EXHIBIT 372

Environmental Pollution 187 (2014) 130–135

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Perinatal multiple exposure to neurotoxic (lead, methylmercury, ethylmercury, and aluminum) substances and neurodevelopment at six and 24 months of age

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ABSTRACT

We studied neurodevelopment in infants from two communities. Children living in the vicinity of tin-ore kilns and smelters – TOKS; $n = 51$) were compared to children from a fishing village (Itapuã; $n = 45$). Mean hair-Hg (HHg) concentrations were significantly higher in Itapuã children which received significantly ($p = 0.0000001$) less mean ethylmercury (88.6 μg) from Thimerosal-containing vaccines (TCV) than the TOKS children (120 μg). Breast-milk Pb concentrations were significantly higher in the TOKS mothers ($p = 0.000017$; 10.04 *vs.* 3.9 μg L^{-1}). Bayley mental development index (MDI) and psychomotor development index (PDI) were statistically significant (respectively $p < 0.0000001$, $p = 0.000007$) lower for the TOKS children only at 24 months of age. Multivariate regression analysis showed that MDI was negatively affected by breast-milk Pb and by HHg. PDI was positively affected by breastfeeding and negatively affected by ethylmercury. Milestone achievements were negatively affected by breast-milk Pb (age of walking) and by HHg (age of talking).

1. Introduction

Early life neurodevelopmental challenges and resulting disabilities due to cumulative exposure to hazardous substances begin at pregnancy and/or during the post-natal period. Additionally, socio-economic disparities associated with psychological stimuli can modulate trajectories that influence mental and psychomotor outcomes. Exposure to environmental neurotoxic substances, *per se* or in combination, can burden the central nervous system (CNS) of the fetus and young child.

Due to the increased pollution or environmental contamination, children nowadays are exposed to more man-made toxic agents than in the past (Landrigan et al., 2005). The number of toxic molecules that are introduced with modern-day manufactured goods (including biocides) has increased considerably. As a result of CNS immaturity, the unborn fetus and infant have to deal with different kinds of toxic substances co-occurring from multiple sources. Neurotoxic metals (*e.g.* lead, mercury, and aluminum) *per se* are known to negatively affect neurodevelopment even at low doses. Indeed, developmental effects have been demonstrated in animal models and have also been observed in children (Rice and Barone, 2000; Carpenter, 2001; Fox et al., 2012). As reviewed elsewhere (Rice and Barone, 2000; Fox et al., 2012), the effects of such substances can be developmental delays, transient or persistent neurological deficits, with neurobehavioral consequences in the individual and societal costs (Bellinger, 2004; Attina and Trasande, 2013). Worldwide, with the increase in manufactured goods and economic globalization, there is a high prevalence of exposure to neurotoxic chemicals *per se* or in combination.

Organic Hg compounds (methylmercury – MeHg, ethylmercury – EtHg) are comparably toxic and hazardous with demonstrable risks shown in animal and human studies (Dórea et al., 2013). While MeHg exposure is mainly through consumption of fish and seafood, EtHg exposure occurs only through Thimerosal-containing vaccines (TCV) widely used in pediatric populations of third-world countries. Additionally, besides EtHg, TCV contains adjuvant-Al (Dórea and Marques, 2010); individually, these substances are below the currently assumed toxicological threshold. However, cumulative

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doses (during frequent immunization in infancy) can attain levels that are of concern (Marques et al., 2007). In the Amazon, when fish is consumed, MeHg is an obligatory dietary component driving exposure and hair Hg (HHg) concentrations in mothers and exclusively breastfed infants (Marques et al., 2013b). During immunization with TCVs, both pregnant mothers and nursing infants are also exposed to EtHg (and adjuvant-Al) (Dórea, 2007; Dórea and Marques, 2010). To deal with concerns about organic Hg exposure, the World Health Organization (WHO) has set guidelines to limit fish-MeHg intake during pregnancy and lactation (JECFA, 2004), but considers the current exposure to multiple doses of TCV-EtHg to be safe (WHO, 2012). While eating fish during pregnancy can have neuroprotective attributes that counteract fish-MeHg effects (Sagiv et al., 2012), exposure to TCVs (during pre- and post-natal period), however, has no co-occurring counteracting substances against putative effects of EtHg (combined with adjuvant-Al).

It is known that neurotoxic chemicals (Pb, MeHg, EtHg, Al) *per se* can reach the CNS causing an adverse effect; however, neurological effects are less well known when these metals occur concomitantly. Usually, when a toxic substance does not show a measurable adverse or untoward effect, the studied level of exposure may be considered safe or without consequence. Despite the vast volume of literature about individually studied neurotoxicity of lead, MeHg, EtHg, and aluminum, it is disappointing how little we know about their co-exposure and combined effects. Therefore, it is important to assess real life perinatal exposure to these metals and their effects on neurodevelopment. Furthermore, early identification of environmental toxic substances and modifying factors of neurological outcomes in children are essential for successful interventions to reduce potential hazards.

The vaccination rate in Brazil is high for the pediatric population, but regional differences are noted (Domingues et al., 2012). In the Amazon region, following the vaccination schedule constitutes a challenge for isolated regions that lack roads and health infrastructure. In a cohort formed from families in the Western Amazon (Marques et al., 2013a), we identified differences in exposure to environmental lead (Marques et al., 2013c). Therefore we took the opportunity to assess neurodevelopment (MDI, PDI, age of walking, and age of talking) between two communities with a distinct pattern of exposure to fish MeHg, TCV (EtHg combined with adjuvant-Al).

The primary objective of this study is to assess early life multiple exposures to low doses of known neurotoxic metals in the Western Amazon aiming to answer two questions: (1) what environmental differences are that may lead to different exposure sources of neurotoxic substances; and (2) how co-occurring substances influence neurodevelopment.

2. Materials and methods

This is part of a large cohort formed to study health related issues in rural and urban populations from low socio-economic backgrounds living in the State of Rondonia (West Amazon), Brazil; this cohort spanned over five years (2007–2012). The study protocol was approved by the Ethics Committee for Studies in Humans of the Federal University of Rondonia (Of. 001-07/CEP/NUSAU). Thus, we compared children living in the vicinity of tin-ore kilns and smelters (TOKS) with children living in a fishing village for multiple exposures to toxic metals (Pb, methylmercury-MeHg, ethylmercury-EtHg, and Al).

The first publication assessed maternal fresh-water fish consumption (as HHg) and birth weight, and detailed the cohort formation (Marques et al., 2013a). Additional publications addressed trans-generational fish-MeHg transfer (Marques et al., 2013b) and breast-milk Pb concentrations in the vicinity of tin-ore smelters (Marques et al., 2013c). This present study focused on the families of the fishing community of Itapuã (Marques et al., 2013a), and those families living in the vicinity of tin-ore processing facilities (smelters in the city of Ariquemes, $n = 31$; kilns in the mining settlement of Bom Futuro, $n = 20$), for which we had data on breast-milk Pb (Marques et al., 2013c). Ariquemes is a city with an industrial district that has several operational tin-smelters that refine concentrated 'cassiterite' ores mined in the nearby Bom Futuro region where raw tin-ore is first processed (Marques et al., 2012) in kilns. These tin-ore smelters and kilns (TOKS) emit metals into the atmosphere. The immediate neighborhood of these TOKS was listed as part of our larger study (Marques et al., 2013a). The village of Itapuã is formed principally by riverines and has been in a previous cross-sectional study of pre-school children (Marques et al., 2011). We assessed multiple exposures (breast-milk Pb, methylmercury-MeHg, ethylmercury-EtHg, and Al) and neurological development (age of walking, age of talking, MDI and PDI at six and 24 months).

The use of biological matrices, such as human milk and hair, are non-invasive (causing minimal discomfort and without risk) and cost-effective means of measuring perinatal exposure to chemical substances. Therefore, population studies of toxicity of MeHg have relied on Hg concentrations measured in hair in relation to neurological symptoms or functional tests applied in children and adults. While this is possible for MeHg derived from habitual fish consumption, the acute exposure and faster metabolism of EtHg (derived from TCV) make it difficult to trace it in hair of infants and children (Dórea et al., 2011). Total Hg in hair represents both MeHg and EtHg. Thus, accurate knowledge about vaccination status which relies on vaccine records made by authorized health professionals is a gold standard for vaccine uptake (Mangtani et al., 2007). During pregnancy and infancy, the recorded TCV dose provides an efficient measure of Thimerosal-EtHg (combined with adjuvant-Al) exposure. Information on TCV was taken from vaccination card records during a visit for neurological assessment.

According to the Brazilian immunization schedule, during the first six months of life, an infant receives cumulative doses of Thimerosal-containing vaccines (TCVs) through hepatitis B, DTP and, depending on immunization efforts, the seasonal anti-flu vaccines. Additionally, pregnant mothers are also targeted for immunization against tetanus (a TCV), which is recommended in a series of three shots (Marques et al., 2007). All these TCVs (Tetanus toxoid, Hepatitis B, and DTP vaccines) are adjuvanted with aluminum.

The TCVs were from the same Brazilian maker (Biomanguinhos, Rio de Janeiro, Brazil) and were formulated to contain a concentration of 2.5 mg mL^{-1} of Al-adjuvant. We adjusted the adjuvant-Al exposures to represent doses of 0.25 mL (0.63 mg Al) of Hepatitis B (taken by infants) and 0.5 mL (1.25 mg Al) for the infants' DTP vaccine and for tetanus toxoid vaccine taken by pregnant mothers. Other Al-adjuvanted vaccines were not used by mothers or infants at the time of the study. For each infant, we computed total EtHg (pre- and post-natal) exposure from each vaccine taken by the mother during pregnancy (Tetanus toxoid) and by the infant during the first six months (Hepatitis B and DTP). The infants received the TCVs of the Brazilian schedule at variable ages depending on their addresses, i.e., if they lived in distant locations or their mothers did not attend pre-natal clinics, there were different intervals between vaccinations; these challenges also account for differences in vaccine coverage (Domingues et al., 2012).

During home-visiting to apply the Bailey tests, we collected a sample of hair according to our standard procedures detailed elsewhere (Marques et al., 2011). Hair samples were cut with stainless-steel scissors, bundled together, and kept in a properly identified envelope until analysis (Institute of Biophysics of the Federal University of Rio de Janeiro). Total Hg determination was done after digestion according to routine procedures described elsewhere (Marques et al., 2013a). Samples of hair were comminuted, washed with EDTA 0.01%, dried in an oven at 50 °C, weighed, and digested with 5 mL of HNO3:H2SO4 (1:1) and 4 mL of 5% KMnO4 using a digestion block at 80 °C for 40 min. Total Hg was then determined by cold vapor atomic absorption spectrometry with a flow injection system (CV-AAS- FIMS; Perkin-Elmer–FIMS 400, Ueberlingen, Germany).

Lead in human milk was quantified as already described in a preceding publication (Marques et al., 2013c). Detailed analysis of total Hg (in hair) concentrations has also appeared in previous publications (Marques et al., 2013a).

Considering the impact of the elevated breast-milk Pb concentrations (Marques et al., 2013c), we addressed milestone achievements (age of walking and age of talking) and neurodevelopment outcomes (MDI and PDI at 6 and 24 months) in comparison with infants from a fishing village far from the TOKS sites.

2.1. Neurodevelopment evaluation and milestone achievements

Neurodevelopment outcomes were measured as milestone achievements (age of walking and age of talking), and through the application of the Bailey scales tests. The ages at which the children first walked or first talked were based on mothers' recollection obtained at the time of visit. The Bayley Scales of Infant Development tests (Bayley, 1969) are based on sets of standardized items that assess personal/social, cognitive, language, and motor development, producing a mental development index (MDI) and a psychomotor development index (PDI); these tests were conducted at the ages of six and 24 months. The Bayley Scale of Infant Development II (BSID-II) was applied and the children's PDI and MDI were calculated; this Bayley-II version is more sensitive for capturing severe motor and cognitive disabilities (Jary et al., 2013). The test was administered in the quiet and familiar atmosphere of home with the mother by the same personnel. Trained psychologists administered the test battery unaware of maternal and infant exposure. Information on breastfeeding practices and socioeconomic data was based on the questionnaire administered to the mothers at the time of the Bayley Scales testing.

2.2. Statistical analysis

We tested for the normality of data distribution using the Kolmogorov–Smirnov one-sample test in order to apply appropriate statistical analysis. In the null-

hypothesis test and linear regression models we used only the variables that had a complete set of data. Group means and medians (birth weight, HHg at 6 months, age of walking, and age of talking, breastfeeding duration) were compared using parametric (*t*-test) and non-parametric (Mann–Whitney test-*U* test, alternative to the *t* test for independent samples) tests as required. For all variables, except for HHg at birth, the sample size was estimated with the entire set of samples ($n = 96$); for the HHg at birth we used $n = 91$ because five newborns (in the TOKS group) did not have sufficient hair mass for the Hg chemical determination. The variables (birth weight, family income, maternal education, breastfeeding length, breast-milk Pb concentrations, infant and maternal hair-Hg) included in the regression model were based on known effects on health and neurodevelopment outcomes. Maternal education entered as a discrete variable, *i.e.*, number of years spent at school. A p value of ≤ 0.05 was accepted as statistically significant for the null hypotheses.

Multiple linear regression analysis (case wise) was used to assess the relationship between neurodevelopment (MDI, PDI, age of walking and age of talking) and maternal and infant variables (birth weight, HHg at 6 and 24 months, TCV-EtHg, age of breast milk Pb concentrations, breastfeeding duration, family income, maternal schooling). We ran the regression model with cohorts (groups) as random factor to assess potential interactions of predicting variables of neurodevelopment. We considered the least acceptable level of significance ($p < 0.05$). Regression coefficients are presented with 95% confidence intervals (CI) of the residuals and the level of significance was $p \leq 0.05$. We also used Pearson Chi-Square to test differences in percent of MDI and PDI <80 between and within (age tested) groups. All statistical analyses were carried out using XLSTAT (Adinsoft, version 1.01, 2013, Paris, France).

The statistical power (the probability of having made a correct decision) was estimated in all cases when $p \leq 0.05$ to confirm that the null hypothesis is true (no real difference between the groups).

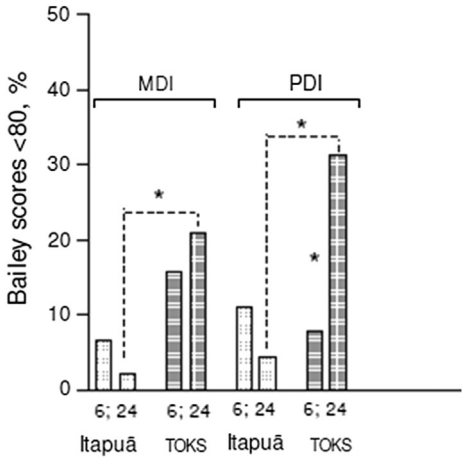

Fig. 1. Changes in Bailey scores <80 from 6 to 24 months in infants from a fishing village (Itapuã) and in those living near tin-ore kilns and smelters (TOKS). *Statistically significant difference for PDI between ages in TOKS children ($p = 0.003$), and between Itapuã and TOKS children at age of 24 months for MDI ($p = 0.004$) and PDI ($p = 0.001$).

3. Results

The main results summarizing exposure and neurodevelopment outcomes of infants from the TOKS areas and from the fishing village (Itapuã) are shown in Table 1. Compared to TOKS, Itapuã children showed significantly higher HHg concentrations at all measured times, birth ($p = 0.024685$), 6 months ($p < 0.0000001$), and 24 months ($p < 0.0000001$). However, at six months of age, Itapuã infants had significantly lower ($p = 0.0000001$) exposure to TCV (EtHg combined with Al) and to breast-milk Pb ($p = 0.000017$). Under this pattern of multiple metal exposure, the MDI was lower for the TOKS children at 6 months ($p = 0.068$) but was statistically significant ($p = 0.0000001$) only at 24 months of age. PDI was also significantly lower ($p = 0.000007$) at 24 months in the TOKS infants than in the group from Itapuã.

The MDI and PDI scores showed different trends during the follow-up from six to 24 months between the two groups of children, i.e., reversibility and aggravation. While the mean scores (for both MDI and PDI) decreased in TOKS children, the opposite trend was seen for the Itapuã ones (Table 1). To further illustrate the asymmetry of neurodevelopment, the proportion of infants with MDI and PDI scores <80 (indicative of mild delay) is shown in Fig. 1. In the Itapuã children only three (6.7%) had MDI scores <80 at six months, and this number decreased to one (2.2%) at 24 months (not statistically significant; $p = 0.308$); however, there were eight TOKS children (15.7%) with MDI scores <80 at six months, a number that increased to 11 (21.6%) at 24 months (not statistically significant; $p = 0.306$). PDI scores <80 in the Itapuã children were five (11.1%) at six months that decreasing to two (4.4%) at 24 months (not statistically significant; $p = 0.217$). For TOKS, however, four children (7.8%) with PDI scores <80 at six months increased to 16 (31.4%) at 24 months; this was statistically significant ($p = 0.003$). Differences in proportion of infants with scores <80 were significantly higher in TOKS children, for both MDI ($p = 0.004$) and PDI ($p = 0.001$), than for Itapuã children.

Table 1
Comparison of infants' exposure and biological parameters between two communities in Western Amazon.[a]

Characteristics	TOKS ($n = 51$)		Itapuã ($n = 45$)		Mann–Whitney *U* test and *t* test
	Median (Min–Max)	Mean (SD)	Median (Min–Max)	Mean (SD)	
Birth					
Weight, kg[b]	3.15 (2.40–4.67)	3.20 (0.46)	3.39 (2.45–5.14)	3.38 (0.48)	0.041895
Hg-hair, μg g^{-1}[c]	1.28 (0.37–3.53)	1.58 (0.91)[b]	1.95 (0.85–5.76)	2.04 (0.99)	0.024685
6 months					
MDI	95 (55–115)	92.25 (14.66)	95 (70–115)	98.22 (12.07)	0.068495
PDI	100 (60–115)	95.53 (11.33)	95 (65–115)	95.44 (13.81)	0.578472
Hg-Hair μg g^{-1}	1.52 (0.67–3.50)	1.69 (0.86)	3.02 (1.04–6.44)	3.02 (1.11)	0.0000001
EtHg-Vaccines, μg	112.5 (12.5–187.5)	119.85 (43.17)	112.5 (0–137.5)	88.61 (39.33)	0.0000001
24 months					
MDI	90 (55–110)	89 (16.58)	111.25 (72.5–115)	106.14 (10.78)	0.0000001
PDI	92 (55–110)	87.10 (17.26)	115 (65–115)	102.81 (14.82)	0.000007
Hg-Hair, μg g^{-1}	2.15 (0.82–4.92)	2.46 (1.08)	4.84 (1.66–9.79)	4.85 (1.64)	0.0000001
Breastfeeding, m[b]	6 (3–24)	8.63 (6.27)	10 (1–24)	12.02 (7.17)	0.004555
Age at talking[b]	12 (10–20)	13.27 (2.60)	13 (10–22)	13.76 (2.64)	0.360421
Age at walking[b]	13 (10–18)	13.22 (2.17)	14 (11–20)	14.71 (2.72)	0.009734
Income, m[b]	700 (00–4050)	857.16 (700.75)	450 (140–4500)	646.33 (683.78)	0.000608
Mother education, y[b]	7 (2–15)	7.27 (3.77)	8 (2–11)	7.53 (2.11)	0.310211
Pb (μg/L)[a]	8.2 (0.9–29.4)	10.04 (8.37)	2.5 (0.7–16.2)	3.89 (3.78)	0.000017

[a] Adapted from Marques et al. (2013a).
[b] Nonparametric distributions.
[c] Mean of 46 samples.

Are differences in MDI and PDI relevant to individual metal exposure or were they confounded due to additional measured socio-environmental stressors? What were the weights of counteracting effects of maternal fish consumption and breastfeeding? The pattern of exposure was not symmetrical between the two communities and a multivariate regression analysis (Tables 2–4) was run with all children combined; significant associations were found.

Table 2 shows regression models displaying the relationships between MDI (at six and 24 months and statistical level of significance, respectively $p = 0.023$ and $p = 0.001$) and variables of interest. At six months, only breast-milk Pb was negatively associated ($p = 0.007$) with MDI ($\beta = -0.29$ [95% CI: −0.50, 0.08]), whereas at 24 months prenatal exposure (HHg at birth) was negatively associated ($p = 0.044$) with MDI ($\beta = -0.36$ [95% CI: −0.71, −0.001]). In Table 3 a summary of multivariate analysis for PDI shows no statistically significant model ($p = 0.172$), but shows a significant association ($p = 0.012$) with breastfeeding ($\beta = 0.287$ [95% CI: 0.06, 0.50]) and TCV ($p = 0.009$) exposure – representing both EtHg plus adjuvant-Al ($\beta = -0.29$ [95% CI: −0.50, −0.07]). In Table 4, both age of walking and age of talking, are shown in relation to the measured variables. Age of talking was affected ($p = 0.028$) by prenatal Hg (HHg) exposure ($\beta = 0.441$ [95% CI: 0.04, 0.83]); age of walking was negatively affected ($p = 0.052$) by breast-milk Pb ($\beta = -0.22$ [95% CI: −0.43, −0.002]), however, the regression model was not significant ($p = 0.221$ and $p = 0.419$ respectively).

Fig. 2 shows total exposure to EtHg and Al in TCV administered to mothers during pregnancy and to infants for both groups. There were only two unvaccinated children, whereas 30 mothers received at least one dose of TCV (tetanus toxoid) during pregnancy. The majority of children (65) showed a full vaccination exposure to TCV (three hepatitis B, and three DTP) according to the immunization schedule. Because some mothers had taken tetanus toxoid vaccine during pregnancy in some cases the infant's exposure to TCVs exceeds six (Fig. 2); most of the TCV occurring during pregnancy also coincided with the infants that were fully vaccinated by the age of six months, i.e., ≥5.

4. Discussion

This study is among the few that integrate positive (breastfeeding, maternal education and fish consumption) factors and negative (simultaneous exposure to neurotoxic metals) stressors of neurodevelopment. An identified pattern of multiple exposures to neurotoxicants (MeHg, Pb, and EtHg combined with Al) differed significantly between two Amazonian groups of children and was significantly associated with neurodevelopmental delays. The TOKS children exposed to higher breast-milk Pb and EtHg (combined with adjuvant-Al from TCV) showed a significantly lower MDI and PDI than the group of infants from Itapuã with consistently higher HHg (birth, six and 24 months). Background maternal body burden of MeHg drove HHg in Itapuã infants; curiously, neurodevelopment (MDI, PDI) delays were less pronounced in these infants.

In infancy, during exclusive breastfeeding, the relatively high intake of breast-milk can lead to substantial exposure to Pb (Marques et al., 2013c) and Hg (Dórea, 2004; Vieira et al., 2013) in Amazonian children. Additionally, during the first months of lactation, exposures to EtHg (combined with adjuvant-Al from TCV) were estimated to be higher than exposures through breast milk (Dórea and Marques, 2010); actually the first hepatitis B vaccine exposes babies at birth to an Al dose (250 mg) five times the total exposure of absorbed Al (55 mg) equivalent to 6 months of breastfeeding (Dórea and Marques, 2010). The additional dose of EtHg derived from hepatitis B and DTP series at two months is equivalent to total Hg exposure of six months of exclusive breastfeeding (Dórea, 2007). Although the quantities of mercury acquired through breastfeeding (enteral MeHg) may be comparable to those in TCVs (parenteral EtHg) they do differ in bioavailability (Harry et al., 2004).

Little is known about multiple exposures to neurotoxic metals during early life and neurodevelopmental effects. Nicolescu et al. (2010) measured concentrations of lead, mercury, and aluminum in blood of 8–12-year-old children, and found that core elements of ADHD were adversely affected by low blood Pb (below 10 μg/dl), but not by mercury or aluminum. In our multiple regression

Table 3
Regression summary for dependent variable: PDI 6 M $R = 0.36$ $R^2 = 0.13$; adjusted $R^2 = 0.04$ F(8; 82) = 1.4994 $p < 0.172$. Regression summary for dependent variable: PDI 24 M $R = 0.47$ $R^2 = 0.22$; adjusted $R^2 = 0.135$ F(9; 81) = 2.2717 $p < 0.012$.

	PDI 6 M (model $p < 0.240$)			PDI 24 M (model $p < 0.019$)		
	Beta	±95%CI	p	Beta	±95%CI	p
Birth weight	−0.063	(−0.28; 0.15)	0.562	−0.048	(−0.25; 0.16)	0.637
Income	−0.002	(−0.24; 0.24)	0.986	−0.078	(−0.30; 0.15)	0.498
Mother education	−0.184	(−0.41; 0.05)	0.117	0.086	(−0.13; 0.30)	0.443
Breastfeeding*	0.287	(0.06; 0.50)	0.012	–	–	–
Breastfeeding**	–	–	–	0.073	(−0.14; 0.29)	0.507
Pb (μg/L)	−0.062	(−0.28; 0.16)	0.576	−0.129	(−0.34; 0.08)	0.223
Hg-hair birth	0.139	(−0.25; 0.52)	0.476	−0.193	(−0.56; 0.17)	0.301
Hg-Hair 6 m	−0.240	(−0.63; 0.15)	0.222	0.107	(−0.35; 0.57)	0.648
Hg-Hair 24 m	–	–	–	0.155	(−0.19; 0.50)	0.382
EtHg-vaccines	−0.026	(−0.26; 0.19)	0.818	−0.288	(−0.50; −0.07)	0.009

Table 2
Regression Summary for Dependent Variable: MDI 6 M ($R = 0.43$ $R^2 = 0.188$); adjusted $R^2 = 0.11$ F(8.82) = 2.2903 $p < 0.0223$ – Regression Summary for Dependent Variable: MDI 24 M ($R = 0.53$ $R^2 = 0.28$; Adjusted $R^2 = 0.20$ F(9.81) = 2.386945; $p < 0.001$).

	MDI 6 M (model $p < 0.023$)			MDI 24 M (model $p < 0.004$)		
	Beta	±95%CI	p	Beta	±95%CI	p
Birth weight	0.139	(−0.06; 0.34)	0.184	0.169	(−0.03; 0.36)	0.090
Family income	−0.061	(−0.29; 0.17)	0.599	−0.009	(−0.23; 0.21)	0.928
Mother education	−0.116	(−0.34; 0.09)	0.303	0.147	(−0.06; 0.36)	0.173
Breastfeeding[a]	−0.121	(−0.34; 0.09)	0.266	–	–	–
Breast milk-Pb	−0.293	(−0.50; 0.08)	0.007	−0.145	(−0.34; 0.06)	0.157
Hair-Hg, birth	−0.234	(−0.60; 0.13)	0.215	−0.355	(−0.71; 0.001)	0.051
Hair-Hg, 6 m	0.128	(−0.24; 0.50)	0.497	0.093	(−0.35; 0.54)	0.679
Hair-Hg, 24 m	–	–	–	0.312	(−0.03; 0.65)	0.071
Vaccines-EtHg	0.042	(−0.17; 0.26)	0.704	−0.151	(−0.36; 0.06)	0.153
Breastfeeding	–	–	–	0.073	(−0.14; 0.28)	0.495

[a] At six months.

Table 4
Regression Summary for Dependent Variable: idade/fala $R = 0.354$; $R^2 = 0.125$; Adjusted $R^2 = 0.028$ F(11.79) = 1.0923 $p < 0.257$. Regression Summary for Dependent Variable: idade/andar $R = 0.40012044$ $R^2 = 0.16009637$ Adjusted $R^2 = 0.04314776$ $F(11.79) = 1.3689$ $p < 0.20421$.

	Age at walking (model $p < 0.221$)			Age at talking (model $p < 0.419$)		
	Beta	±95%CI	p	Beta	±95%CI	p
Birth weight	0.009	(−0.20; 0.22)	0.932	−0.019	(−0.23; 0.19)	0.857
Income	0.062	(−0.18; 0.30)	0.610	−0.235	(−0.47; 0.001)	0.058
Mother education	0.010	(−0.22; 0.24)	0.932	0.087	(−0.15; 0.32)	0.466
Breastfeeding 6M	0.114	(−0.11; 0.34)	0.315	−0.056	(−0.28; 0.17)	0.625
Pb (μg/L)	−0.219	(−0.43; 0.002)	0.052	−0.066	(−0.28; −0.16)	0.557
Hg-hair birth	−0.158	(0.54; 0.23)	0.420	0.441	(0.04; 0.83)	0.028
Hg-Hair 6 m	0.290	(−0.1; 0.68)	0.143	−0.268	(−0.66; 0.13)	0.181
EtHg-vaccines	0.098	(−0.12; 0.32)	0.390	−0.099	(−0.33; 0.13)	0.389

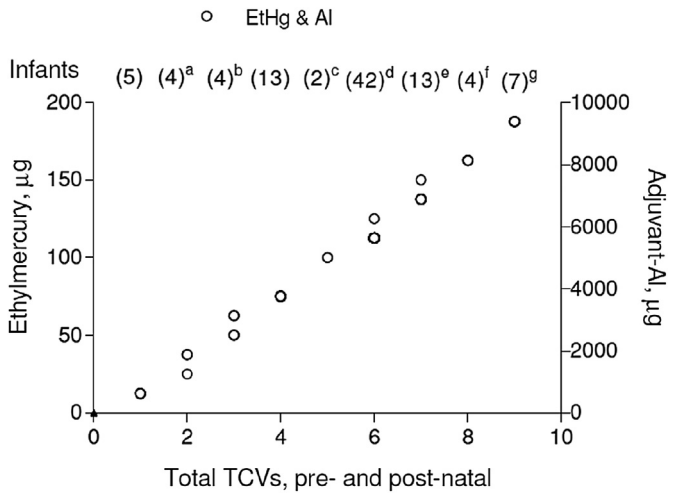

Fig. 2. Total Thimerosal-containing vaccine (TCV) exposure and respective loads of ethylmercury (EtHg) and adjuvant-Al. Circles represent doses of both EtHg and Al in TCVs administered during pregnancy and in infancy. Number of infants (n) for corresponding total TCV doses received are shown in upper row; superscript letters indicate the number of infants that were exposed to maternal TCV during pregnancy: $a = 1$, $b = 2$, $c = 2$, $d = 1$, $e = 13$, $f = 4$, $g = 7$.

models, only variables related to maternal exposure during pregnancy (milk-Pb and HHg at birth) showed significant association (negative) with MDI at 24 months; however, for PDI at 24 months, significant negative associations were seen in relation to HHg at birth and EtHg (combined with Al). Not only gestational exposures from maternal body burden (mercury and lead), but also TCV (EtHg and Al) showed significant differences in exposure between the groups of children (Table 1).

Studies in European settings, where HHg concentrations resulting from fish consumption are much lower than in Amazonians, addressed Hg exposure from TCV-EtHg (Heron et al., 2004), fish-MeHg (Hibbeln et al., 2007; Daniels et al., 2004), and amalgam-Hg (Daniels et al., 2007) in a series of independent publications. Collectively these studies seem to indicate that both maternal fish intake during pregnancy and fish intake by children were associated with higher mean neurobehavioral scores (references in Dórea et al., 2014). Additionally, results addressing only the EtHg exposure in this ALSPAC cohort were not consistent with organic mercury toxicity (Dórea, 2008; Dórea et al., 2014).

Neurodevelopmental studies addressing multiple exposures that included EtHg are scarce, but they suggest neurological delays in early childhood (Mrozek-Budzyn et al., 2012; Marques et al., 2012; Lee and Ha, 2012; Dórea et al., 2012, 2014). In cross-sectional studies of pre-school children in rural (Marques et al., 2011) and urban (Marques et al., 2012) settings we have not seen clear neurodevelopment effect of fish-MeHg or TCV-EtHg. However, age-specific comparison (six-month-old infants) of neurodevelopment outcomes between rural versus urban settings suggested an association with TCV-EtHg (Dórea et al., 2012). In the present work (*i.e.*, a more rigorous prospective cohort), the findings of strong association between multiple exposures (Pb and EtHg) and neurological outcomes are revealing. Studies addressing simultaneous exposure to organic Hg (which included TCV-EtHg) and other neurotoxic substances showed significant interactions with neurodevelopment in Poland (Mrozek-Budzyn et al., 2012), and in Korea (Lee and Ha, 2012).

While it is relatively simpler to conceive experimental models (animal and cell studies) to measure intended outcomes associated with the toxic level of a target substance (alone or in combination), health scientists are challenged by the limitations of observational studies. It has been largely ignored that early life exposure to multiple neurotoxic substances can occur, albeit in small doses, for which additive and synergistic effects are unknown. In the present work, these challenges were compounded by exposure-associated differences. Therefore, the strengths and weaknesses of this study are intertwined and inseparable: a) exposure route and duration differed between MeHg and Pb (chronic, enteral, and independently or unassociated) and EtHg (acute, parenteral, and in combination with adjuvant-Al); b) no traceable biomarker – parenteral (infant's TCV); *c*) infant's exposure to Pb, however, is most probably derived from both direct atmospheric Pb (from kiln and smelter emissions), and intrinsic maternal-Pb accumulated in bones; d) unaccountable differences in the metabolism of injected Al hydroxide and Al phosphate that may end up in the brain (Hem, 2002). Furthermore, in follow-up studies, modifying factors (positive and negative) can occur and may introduce confounding related to neurodevelopment outcomes. Nevertheless, this work has the unique feature of addressing intergenerational and neonatal exposure to multiple neurotoxicants and neurodevelopment.

The chain of events leading to neurotoxicant exposures during pregnancy and lactation depends on preventable actions. Counter measures for untoward effects in the subclinical level are challenging (Trasande et al., 2006), but attenuating circumstances related to breastfeeding (Dórea, 2007) and maternal education (Marques et al., 2012) are demonstrable. Therefore, it is crucial to understand the effects of multiple exposures to low doses of toxic substances in vulnerable populations in order to ascertain new paradigms for risk analysis.

In this study, we demonstrate that the early development stages (pregnancy and lactation) require consideration of both, special features of exposure and effect modifying factors (Dórea, 2007). The results shed light on complex interactions between positive (breastfeeding duration, socio-educational characteristics of mothers) and negative (exposure to multiple neurotoxicants) factors that influence neurodevelopment. Since early 1990 when countries started to withdraw Thimerosal as a preservative in pediatric vaccines (Wigzell, 1990), studies have shown that Thimerosal-free vaccines are equally effective (Nolan et al., 2009). The present study suggests that Thimerosal-free vaccines should be used to avoid further exposure to additional organic Hg (and adjuvant-Al) besides other co-occurring neurotoxic substances from environmental sources. This alternative form of immunization should be added to the list of indications to protect sensitive individuals and attenuate the risks of higher combined exposure to environmental pollutants.

5. Conclusions

Two groups of young children, with distinct patterns of neurotoxic metal exposures, showed significant differences in neurodevelopmental outcomes. Children with higher exposure to maternal Pb and EtHg showed to be more sensitive to neurodevelopmental delays. The situations of multiple exposures to low doses of neurotoxic metals are complex to analyze, but show a dominant cause of neurodevelopmental delays that can lead to reversibility or aggravation.

Acknowledgments

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EXHIBIT 373

STATE OF MICHIGAN
IN THE CIRCUIT COURT FOR THE COUNTY OF OAKLAND
FAMILY DIVISION
- - -

LORI MATHESON, f/k/a LORI ANN SCHMITT, Plaintiff,	:	
vs.	:	CASE NO. 2015-831539-DM
MICHAEL SCHMITT, Defendant.	:	

VIDEOTAPED DEPOSITION OF STANLEY A. PLOTKIN, M.D.
New Hope, Pennsylvania
January 11, 2018

Reported by:
Maureen Broderick, RPR
JOB NO. 135522

vaccines, how many fetuses have been part of that work?

"A. My own personal work? Two.")

BY MR. SIRI:

Q So I'm going to ask that question again. In your work related to vaccines, how many fetuses were involved in that work?

A There were only two fetuses involved in making vaccines. When fetal strains of, fibroblast strains were first developed, I was involved in that work trying to characterize those cells; but they were not used to make vaccines.

Q Wasn't the purpose of this study to help develop a human cell line or to support the use of human cell lines in the creation of vaccines?

A The idea was to study the cell strains from fetuses to determine whether or not they could be used to make vaccines.

Q So this was related to your work?

A Well, yes, in a sense --

Q To vaccines, correct?

A Yes. It was preparatory.

Q So this study involved 74 fetuses,

correct?

A I don't remember exactly how many.

Q If you turn to page 12 of the study.

A Seventy-six.

Q Seventy-six. And these fetuses were all three months or older when aborted, correct?

A Yes.

Q And these were all normally developed fetuses, correct?

A Yes.

Q Okay. These included fetuses that were aborted for social and psychiatric reasons, correct?

A Correct.

Q What organs did you harvest from these fetuses?

A Well, I didn't personally harvest any, but a whole range of tissues were harvested by co-workers.

Q And these pieces were then cut up into little pieces, right?

A Yes.

Q And they were cultured?

A Yes.

Q Some of the pieces of the fetuses were

pituitary gland that were chopped up into pieces to --

A Mm-hmm.

Q Included the lung of the fetuses?

A Yes.

Q Included the skin?

A Yes.

Q Kidney?

A Yes.

Q Spleen?

A Yes.

Q Heart?

A Yes.

Q Tongue?

A I don't recall, but probably yes.

Q So I just want to make sure I understand. In your entire career -- this was just one study. So I'm going to ask you again, in your entire career, how many fetuses have you worked with approximately?

A Well, I don't remember the exact number, but quite a few when we were studying them originally before we decided to use them to make vaccines.

Q Do you have any sense? I mean, this one study had 76. How many other studies did you have that you used aborted fetuses for?

A I don't remember how many.

Q You're aware, are you aware that the, one of the objections to vaccination by the plaintiff in this case is the inclusion of aborted fetal tissue in the development of vaccines and the fact that it's actually part of the ingredients of vaccines?

A Yeah, I'm aware of those objections. The Catholic church has actually issued a document on that which says that individuals who need the vaccine should receive the vaccines, regardless of the fact, and that I think it implies that I am the individual who will go to hell because of the use of aborted tissues, which I am glad to do.

Q Do you know if the mother's Catholic?

A I have no idea.

Q Okay.

A But she should consult her priest.

Q If she has a -- if she's, in fact, Christian, I guess, right?

In any event, so we have 76 in this study. Would you approximate it's been a few

hundred fetuses?

A Oh, no, I don't think it was that many. Probably not many more than in this paper.

And I should stipulate that we had nothing to do with the cause of the abortion.

Q Some of these were for psychiatric institutions, correct?

A Actually, all I can say is that the fetuses that I personally worked with actually came from Sweden, from a Swedish co-worker. And so I, in no case, was able to determine what exactly the reason for the abortion was.

Q I'm just asking you, some of the fetuses that you did use did come from abortions from people who were in psychiatric institutions, correct?

A I don't know that. What I'm telling you is that I got them from a co-worker; and if it's stated in the paper, it's true. But, otherwise, I do not know.

Q So if it's in the paper, you don't contest it, right?

A I don't contest it, no.

Q Okay. Have you ever used orphans to study an experimental vaccine?

A Yes.

Q Have you ever used the mentally handicapped to study an experimental vaccine?

A I don't recollect ever doing studies in mentally handicapped individuals. At the time in the 1960s, it was not an uncommon practice.

Q So you're saying -- I'm not clear on your answer. I'm sorry. Have you ever used mentally handicapped to study an experimental vaccine?

A What I'm saying is I don't recall specifically having done that, but that in the 1960s, it was not unusual to do that. And I wouldn't deny that I may have done so.

(Discussion off the stenographic record.)

BY MR. SIRI:

Q I'm going to read you a sentence from what what's been previously marked as --

MS. RUBY: No, that wasn't.

BY MR. SIRI:

Q -- Exhibit 7.

MS. RUBY: That's not what got marked as Exhibit 7. That got -- the task force was seven.

MR. SIRI: Oh.

MS. NIEUSMA: So this should be 42.

MR. SIRI: Got it. Got it. Got it.

(Exhibit Plaintiff-42 was marked for identification.)

BY MR. SIRI:

Q Well, in any event, you're not denying that you, that you -- well, there's an article entitled "Attenuation of RA 27/3 Rubella Virus in WI-38 Human Diploid Cells." Are you familiar with that article?

A Yes.

Q In that article, one of the things it says is 13 -- is one of the things it says is: 13 seronegative mentally retarded children were given RA 27/3 vaccine?

A Okay. Well, then that's, in that case that's what I did.

Q Have you ever expressed that it's better to perform experiments on those less likely to be able to contribute to society, such as children with handicap, than with children without or adults without handicaps?

A I don't remember specifically, but it's

EXHIBIT 374

HEALTH NEWS MAY 2, 2012 / 4:45 AM / 8 YEARS AGO

GAVI man's mission to "immunize every kid on earth"

Kate Kelland

6 MIN READ

ACCRA (Reuters) - Seth Berkley was a young epidemiologist working for the U.S. State Department when he saw the graves left behind after measles swept through refugee camps in Sudan during the 1985 famine.

Chief executive of the Global Alliance for Vaccines and Immunisation (GAVI), Seth Berkley, poses for a photograph with children in the village of Amanfro April 27, 2012. Berkley's interim goal with GAVI is to save 4 million lives by 2015, and his big mission is for the global health community to get vaccines against every preventable disease to every child who needs protecting. The Alliance, set up in 2000, uses private and government donor backing to negotiate down vaccine prices for the developing world and then bulk-buy and deliver them to countries whose populations need them most. In its first decade, GAVI says it has already financed immunisation that has prevented more than 5.5 million premature deaths from common but life-threatening diseases. Picture taken April 27, 2012. REUTERS/GAVI/2012/Olivier Asselin/Handout

"You'd see little shallow graves, lined up, one after the other - babies. That's what happens when measles goes through a nutritionally deficient community. It's a horrible disease and it spreads incredibly efficiently," he says.

Now, as chief executive of the Global Alliance for Vaccines and Immunization (GAVI), Berkley's specialism is vaccinology and he is in Africa again, working to introduce routine childhood immunizations which protect most people in the rich world.

Here in Ghana, there have been no deaths from measles since 2003, and no cases of polio, another vaccine-preventable disease, since 2008. But

Berkley's sights are set high.

His interim goal with GAVI is to save another 4 million lives by 2015, and his big mission is for the global health community to get vaccines against every preventable disease to every child who needs protecting.

"I wish we could have state-of-the-art hospitals in every corner of the earth...but realistically it's going to be a while before that can happen," he said in an interview.

"But we can immunize every kid on earth, and we can prevent these diseases. It's only a matter of political will, a little bit of money and some

systems to do it."

GAVI, set up in 2000, uses private and government donor backing to negotiate down vaccine prices for the developing world and then bulk-buy and deliver them to countries whose populations need them most.

In its first decade, GAVI says it has already financed Immunization that has prevented more than 5.5 million premature deaths from common but life-threatening diseases.

After teetering on the brink of a funding crisis in late 2010, the group held a pledging conference in London last June and enlisted the help of billionaire philanthropist Bill Gates and the British government to squeeze other donors hard.

The result was $4.3 billion in pledges, substantially more than the $3.7 billion GAVI had asked for and enough to keep the alliance's programs in more than 70 of the world's poorest countries funded until 2016.

Berkley says GAVI's success lies in the sheer size of the market it has created, making it hard for pharmaceutical companies to ignore.

"The concept of GAVI was to create a market for the entire developing world. When we're in negotiations with companies, it's not just about the Togo market or the Ghanaian market, it's about the entire market," Berkley says. "Our birth cohort is 75 million children. That's a big market."

PRICES FORCED DOWN

Since 2000, Big Pharma has gradually seen the prices of its vaccines - many of which cost $70, $90 or even several hundred dollars in the west - forced down in the developing world under pressure from GAVI's market.

Last year a raft of drugmakers including GlaxoSmithKline, Merck, Johnson & Johnson's Crucell and Sanofi-Aventis' Sanofi Pasteur said they would cut their prices on vaccines against diseases such as measles, diarrhoea and meningitis to help GAVI sustain its supplies to the world's poorest countries.

The price GAVI pays for pentavalent vaccines, which protect against diphtheria, tetanus, pertussis, hepatitis B, and Haemophilus influenza type b, was also cut by the India-based firms Serum Institute and Panacea Biotec, bringing it to well below $2.00 per dose.

Slideshow (7 Images)

Yet GAVI is often criticized, in particular by the international medical charity Medecins Sans Frontieres (MSF), which says it still pays too much for some of its vaccines - effectively handing a sizeable profit and a guaranteed market to Western pharmaceutical giants like Pfizer and its British rival GlaxoSmithKline.

Pfizer and GSK signed a 10-year deal with GAVI in March 2010 to supply their patented pneumonia vaccines at a discounted price of $7 per dose for the first 20 percent and $3.50 for the remaining 80 percent - a price MSF said was too high.

"Could the price be cheaper? Probably," Berkley says. "But between the time when we roll this out - now - and the time when there is possibly going to be more competition in the market, 500,000 children would have died of pneumococcal disease.

"So what do you do, just wait and let that happen?"

Berkley admits his determination is "almost like a religious belief", but insists it is also pragmatic.

From a childhood in New York where he talked his way into helping out at a retail chemistry supply store to get closer to the science, he later worked in a ghetto clinic in Mississippi, and moved from there to study tropical medicine in Brazil. He took his first African trip to Senegal in West Africa.

"I love science and I believe in it. I have a faith that science can solve

problems and make the world a better place," he says.

Asked about fears that a focus on Immunization might take attention and funds away from other areas of health, such as building hospitals and improving access to treatment, Berkley says Immunization is simple, cheap and very cost-effective.

"You can't stop wars to build tertiary teaching hospitals, but you can say 'let's stop for a couple of days to immunize the kids'. It has been done," he says.

Reporting by Kate Kelland; Editing by Myra MacDonald

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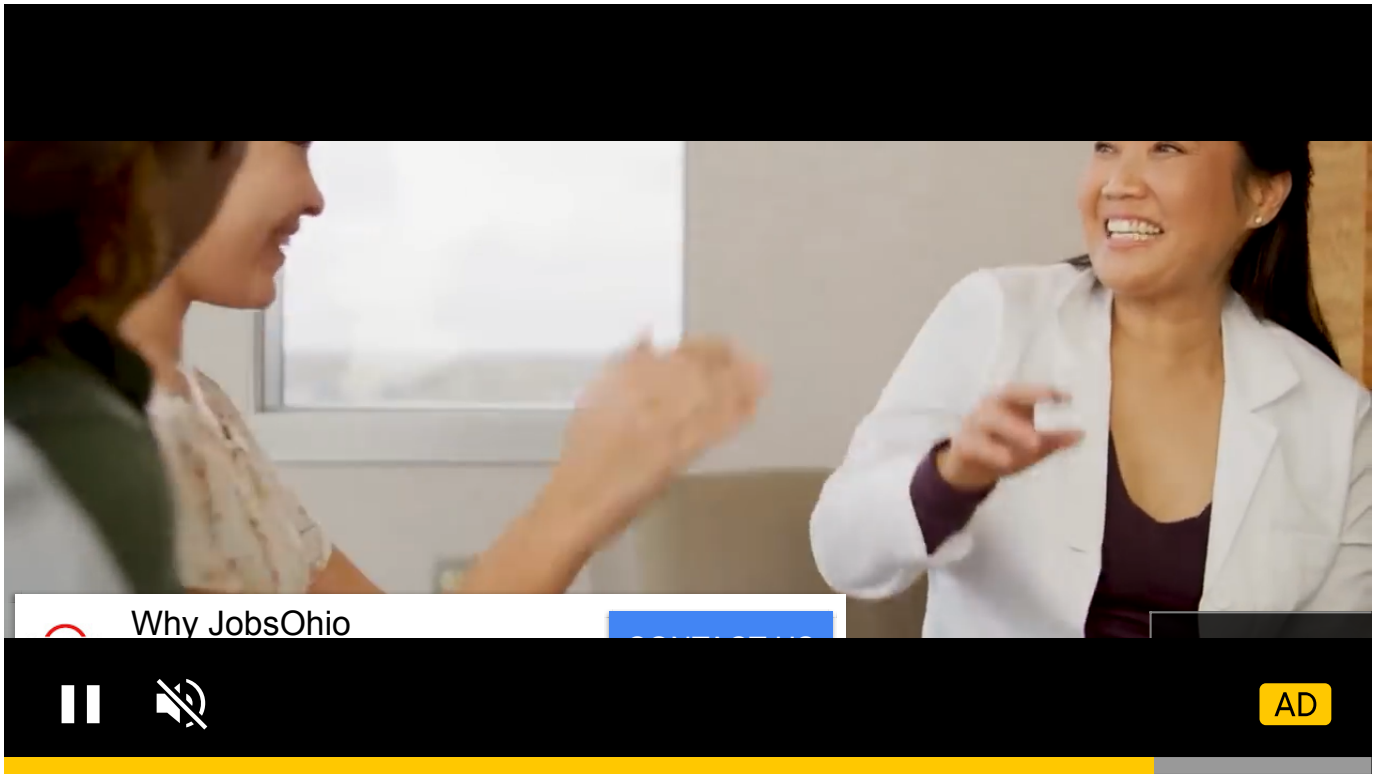

EXHIBIT 375

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION

Advisory Committee on Immunization Practices (ACIP)

Summary Report
June 20-21, 2018
Atlanta, Georgia

clue? Do you know each and every time a baby shot is due? You may have questions. Your doc or nurse will explain. Though the names and timing have changed, the importance is still the same. For protection against serious disease, it's wise to immunize. Complete the full schedule on time and your kids will lead healthier lives."

Dr. Bennett thanked everyone and said she could not begin to tell them all how much this meant and how sad she was to be finishing her term. However, she noted that it was time for "new blood" and that Dr. Romero would do a fantastic job leading them forward. She shared a picture of the Temple of Vaccinia in England at the home of Edward Jenner, who gave cowpox vaccine:

She visited there about six months before she took over as Chair. The people she was visiting in Bristol had no idea she served on the ACIP or was becoming Chair. They coincidentally took her to Jenner's house, which was a wonderful event. Dr. Bennett said the reason she was showing this photograph was because she wanted to reflect for a moment on the work that ACIP does and the fact that it has such a long and amazing history, of which they are all a part. One reason this position has meant so much to her is because she felt like she could play a tiny role in the progression of vaccine science and immunization in this country. It is an incredible history, one that means so much to her, and one in which all members play a critical role.

She emphasized what a huge honor it had been to serve on the ACIP and participate in the decision-making they engage in every time they meet. She thanked the members, liaisons, *Ex Officios,* and especially CDC staff for how much she has learned from them. The other part of this position that was amazing to her was "standing on the shoulder of giants" like Dr. Carol Baker and others before her, from whom she learned every day. She expressed her deep gratitude for being given this opportunity.

EXHIBIT 376

BBC NEWS

Superbug vaccine 'shows promise'

A vaccine to guard against hospital superbug MRSA is a step closer, according to scientists.

US researchers have developed a vaccine that protected mice from four potentially deadly strains of MRSA.

Writing in the Proceedings of the National Academy of Sciences, the team said the study could lead to a human vaccine, though more work was needed.

Methicillin-resistant Staphylococcus aureus often strikes in hospitals where patients' immune systems are weak.

> **Making a vaccine is a bit like witchcraft - you really need to put stuff in, stir the pot round and then see what happens**
> Dr Mark Enright, Imperial College

It is difficult to fight because it has developed a resistance to certain antibiotics.

The team looked for a vaccine using a technique called "reverse vaccinology", which builds on recent genetics advances.

It involved sifting through the genome of Staphylococcus aureus to hunt for proteins on the microbe that might spark the body's immune system into action, producing protection against the bacteria.

The team identified four proteins that prompted a strong immune response, making them good targets for vaccines.

Understanding the mechanism

When they combined the different proteins and injected them into laboratory mice, they discovered the mice had gained protection against five different strains of Staphylococcus aureus.

Four of these strains were MRSA, while the other was a strain associated with toxic shock after burn injuries.

The vaccine gave the mice between 60% and 100% immunity.

The team said further tests would be needed to understand the mechanism of the vaccine and to discover if it would be as effective in humans.

Lead researcher Olaf Schneewind, professor of microbiology at the University of Chicago, said: "This microbe's ability to acquire the tools it needs to protect itself from the drugs we use to treat it is legendary, which is why a vaccine has become such a high priority.

Exhibit 376

"One by one, this organism has learned how to evade nearly all of our current antibiotics. So, generating protective immunity against invasive S aureus has become an important goal."

More research needed

But Dr Jodi Lindsay, senior lecturer at the Centre of Infection at St George's, University of London, said: "I think this study has not discussed some important things.

"The researchers did not look at a good cross-section of strains of MRSA - there are several families of MRSA, each containing many different strains, and the team only looked at a few US-specific strains."

She added that, for a vaccine to be efficient across a number of different strains, the proteins used must be the same in every strain but the proteins used in the experiments varied.

"It is also important to remember a mouse model is not the same as a human model. The hard bit is getting a vaccine from a mouse to a human, because that involves a lot of patients, a lot of money, a lot of regulation, and so far, the best examples have failed."

Dr Mark Enright, leader in molecular epidemiology at Imperial College, London, agreed but said the study was a good starting point.

"Making a vaccine is a bit like witchcraft - you really need to put stuff in, stir the pot round and then see what happens. And you only really know what happens when you try it out in patients and humans."

Story from BBC NEWS:
http://news.bbc.co.uk/go/pr/fr/-/2/hi/health/6098210.stm

Published: 2006/10/31 00:11:44 GMT

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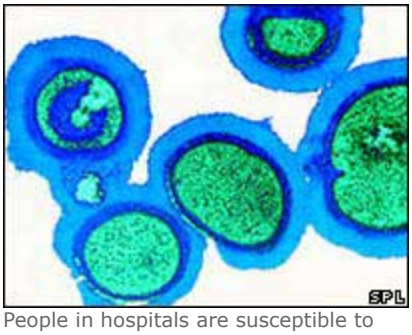
Superbug vaccine 'shows promise'

A vaccine to guard against hospital superbug MRSA is a step closer, according to scientists.


People in hospitals are susceptible to MRSA infection

US researchers have developed a vaccine that protected mice from four potentially deadly strains of MRSA.

Writing in the Proceedings of the National Academy of Sciences, the team said the study could lead to a human vaccine, though more work was needed.

Methicillin-resistant Staphylococcus aureus often strikes in hospitals where patients' immune systems are weak.

It is difficult to fight because it has developed a resistance to certain antibiotics.

> **"This microbe's ability to acquire the tools it needs to protect itself from the drugs we use to treat it is legendary"**
>
> Olaf Schneewind

The team looked for a vaccine using a technique called "reverse vaccinology", which builds on recent genetics advances.

It involved sifting through the genome of Staphylococcus aureus to hunt for proteins on the microbe that might spark the body's immune system into action, producing protection against the bacteria.

The team identified four proteins that prompted a strong immune response, making them good targets for vaccines.

Understanding the mechanism

When they combined the different proteins and injected them into laboratory mice, they discovered the mice had gained protection against five different strains of Staphylococcus aureus.

Four of these strains were MRSA, while the other was a strain associated with toxic shock after burn injuries.

The vaccine gave the mice between 60% and 100% immunity.

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The team said further tests would be needed to understand the mechanism of the vaccine and to discover if it would be as effective in humans.

Lead researcher Olaf Schneewind, professor of microbiology at the University of Chicago, said: "This microbe's ability to acquire the tools it needs to protect itself from the drugs we use to treat it is legendary, which is why a vaccine has become such a high priority.

"One by one, this organism has learned how to evade nearly all of our current antibiotics. So, generating protective immunity against invasive S aureus has become an important goal."

More research needed

But Dr Jodi Lindsay, senior lecturer at the Centre of Infection at St George's, University of London, said: "I think this study has not discussed some important things.

"The researchers did not look at a good cross-section of strains of MRSA - there are several families of MRSA, each containing many different strains, and the team only looked at a few US-specific strains."

She added that, for a vaccine to be efficient across a number of different strains, the proteins used must be the same in every strain but the proteins used in the experiments varied.

"It is also important to remember a mouse model is not the same as a human model. The hard bit is getting a vaccine from a mouse to a human, because that involves a lot of patients, a lot of money, a lot of regulation, and so far, the best examples have failed."

EXHIBIT 378

Press Releases

Rep. Nadler Statement Condemning Trump Administration for Refusing to Lift Fetal Tissue Ban for COVID-19 Vaccine

Research

Washington, March 19, 2020

Tags: *Health Care*, *Trump*, *COVID-19*

Today, Congressman Jerrold Nadler (D-NY) issued a statement condemning the Trump Administration's refusal to lift their ban on fetal tissue research as scientists and medical professionals work tirelessly to develop a COVID-19 vaccine and save lives:

"Scientists have told Congress again and again that fetal tissue is the gold standard for vaccine research. The ban on this research imposed by the Trump Administration last year has no scientific purpose, with reports showing it already impacting promising, life-saving medical research. This new report reveals that even in the midst of a worldwide pandemic, the Trump administration is unwilling to set aside their dangerous anti-science bias to allow research to go forward simply because it involves fetal tissue.

"That the Trump Administration prioritizes their illogical and harmful anti-science agenda over stopping this pandemic shows once again that they would rather score political points with their anti-abortion friends than save millions of lives. The fact that Health and Human Services (HHS) has not even responded to scientists' repeated requests to move forward with this research should horrify every American waiting for a vaccine to COVID-19.

"HHS must authorize this research immediately and while the ban on fetal tissue research should be lifted permanently, at the very least it must be lifted during this pandemic."

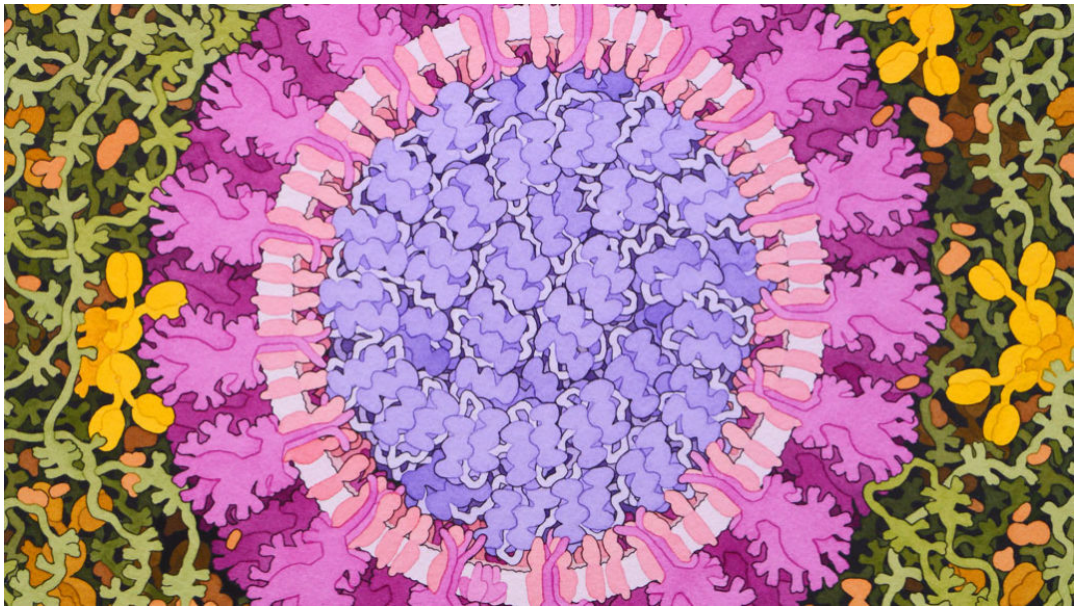
EXHIBIT 379

ScienceNews
INDEPENDENT JOURNALISM SINCE 1921

NEWS HEALTH & MEDICINE

To tackle the new coronavirus, scientists are accelerating the vaccine process

Researchers are turning to nontraditional approaches to create vaccines and therapeutics



The coronavirus that causes COVID-19 (seen in this artist's representation) has inspired scientists to take creative approaches to making vaccines.

DAVID S. GOODSELL, RCSB PROTEIN DATA BANK

By Tina Hesman Saey

FEBRUARY 21, 2020 AT 10:56 AM

As a mystery illness started spreading in China in late December, researchers at Inovio Pharmaceuticals were keeping a close eye on what was happening, even before anyone knew the cause was a coronavirus.

The company, based in San Diego, is no stranger to the viruses. After MERS, which is caused by a different coronavirus, emerged in 2012, Inovio was one of the first to develop a still-experimental vaccine for the disease. In the new outbreak, as soon as Chinese researchers posted the genetic makeup of the virus, dubbed SARS-CoV-2, the company's scientists sprang into action.

"We'd all hoped that there would be enough overlap that our previously

See all our coverage of the coronavirus outbreak

developed MERS vaccine would be helpful in this case," says Kate Broderick, Inovio's senior vice president for research and development. Like MERS and SARS, the new virus is a coronavirus that uses RNA as its genetic material.

But in-depth analysis revealed that the two coronaviruses are too different for a vaccine against MERS, also known as Middle East respiratory syndrome, to take down the new virus. So the company's researchers set about designing a new vaccine.

That design relies on a relatively new approach to vaccine creation, one that the researchers used to develop the MERS vaccine. Traditional vaccines are composed of weakened or killed forms of viruses or parts of viruses, including purified proteins. When injected into a person, the immune system recognizes the virus as an invader and produces antibodies to stave off future invasions. But growing enough debilitated viruses or purifying enough proteins to make vaccine doses for millions of people can take months or even years.

So Inovio and other companies have developed ways to make vaccines much more quickly. For their SARS-CoV-2 vaccine, Inovio scientists convert the virus's RNA into DNA and select pieces of the virus that computer simulations have suggested will prod the immune system into making antibodies. Those selected bits of DNA are then inserted into bacteria, which produce large quantities of protein snippets to be used in the vaccine. This approach drastically shortens the time it takes to make a vaccine. A traditional vaccine takes two to three years to develop. For Inovio's product, it took three hours to design and about a month to manufacture, Broderick says.

Inovio started testing the vaccine in animals at the beginning of February and hopes to begin safety tests in people by early summer.

Even so, Inovio's vaccine is still at least a year away from being widely used. As the number of cases of the novel coronavirus disease, or COVID-19, continues to rise, several other groups are also racing to develop vaccines and therapeutics that take nontraditional approaches to fight the virus.

Novel vaccines for a novel coronavirus

Researchers at the U.S. National Institute of Allergy and Infectious Diseases, working with the Cambridge, Mass.–based biotechnology company Moderna, are developing a messenger RNA, or mRNA, vaccine that will stimulate the body to produce vaccine components. Messenger RNAs are copies of protein-making instructions encoded in the DNA of genes. Cellular machinery reads the mRNA instructions to build proteins.

Scientists have selected portions of SARS-CoV-2 that may spark a vigorous immune reaction against the virus, says Kizzmekia Corbett, a viral immunologist at the NIAID's Vaccine Research Center in Bethesda, Md. The mRNA vaccine will

tell human cells which viral proteins to make, she says.

"We're literally giving the cells a genetic code of our vaccine design, delivered as RNA that will tell cells, 'Hey, make this protein,'" says Corbett, who is the scientific lead on the center's effort to develop the vaccine.

Those proteins — Corbett wouldn't say which viral proteins — will then prod the immune system to make antibodies to protect against the virus. Since the body does all of the protein-production work with the mRNA vaccine, researchers can skip the time-consuming and costly step of manufacturing vaccine proteins.

This strategy could be used to design vaccines against future coronaviruses or other emerging infectious diseases, Corbett says. "What we feel we have developed is a universal strategy, being able to quickly deploy a vaccine if another novel coronavirus should pop up," Corbett says. Other mRNA vaccines against MERS and other diseases are still in the testing phase.

Corbett would not specify a timeline for her team's mRNA vaccine, but Anthony Fauci, director of NIAID, has said the mRNA vaccine could be ready for initial safety testing within months. But the researchers have yet to find a pharmaceutical company to manufacture the large quantities of mRNA doses that would be necessary for use by the general public, Fauci said February 11 in Washington, D. C., at a discussion of the new coronavirus at the Aspen Institute, a nonprofit organization.

Inovio's experience with its MERS vaccine is one example of just how long it typically takes to make sure a vaccine is safe and effective. Inovio conducted initial safety testing of the MERS vaccine in a Phase I clinical trial from February 2016 to May 2017. There were no serious side effects among the 75 healthy adult participants, the researchers reported in 2019 in the *Lancet Infectious Diseases*. The vaccine moved into a Phase II trial in August 2018 to test safety in a larger number of people and determine whether the vaccine spurs the immune system to make protective antibodies. That trial is expected to wrap up later this year.

Even if everything goes swimmingly, the MERS vaccine must still pass Phase III safety and effectiveness testing before being considered for approval by the U.S. Food and Drug Administration. It's the same gauntlet that all new vaccines and drugs must run.

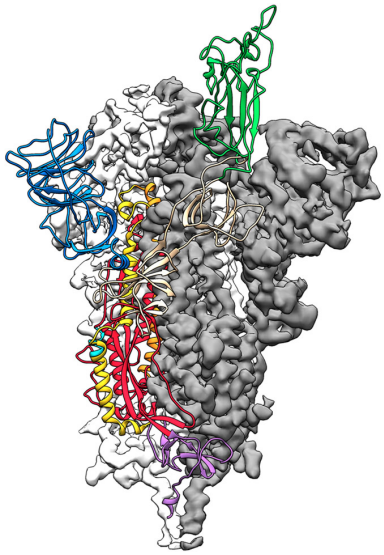
Inovio and the NIAID/Moderna partnership have both received funding from the Oslo-based Coalition for Epidemic Preparedness Innovations. CEPI is also funding yet another type of novel vaccine development. CEPI and researchers from the University of Queensland in Brisbane, Australia, have found a way to clamp down on the coronavirus to keep it from infecting cells.

The Queensland group had already been working with CEPI on molecular clamp vaccines against other viruses for about a year, says Trent Munro, a biotechnologist involved in the work. A molecular clamp is a protein stitched

onto another protein, in this case the coronavirus' spike protein. With SARS and MERS, spike proteins work a bit like malleable lock picks, changing shape to interact with a protein on the surface of human cells and gain entry into them. The 3-D structure of SARS-CoV-2's spike protein, reported online February 19 in *Science*, confirms the protein is also a shape-shifter. But the new coronavirus' spike protein clings 10 to 20 times as tightly to its target on human cells as the SARS version does. Holding on tighter may help the new virus spread more easily from person to person, researchers say.

The molecular clamp the Queensland team devised keeps the spike protein from shape-shifting, locking it in a form that triggers antibody production and thus making it a potent vaccine, Munro says.

The team uses mammalian cells to produce the vaccine, and a specialized machine determines which cells are churning out clamped protein. With the machine, researchers can "do things that would have taken weeks before in just days," Munro says. Laboratory testing may start within weeks. Safety testing in people may begin in months, but it will take much longer for the vaccine to be ready for general use. When the Queensland group began working with CEPI to develop a molecular clamp vaccine, "we thought it would take three years as a test case," Munro says. But the emergence of the new coronavirus forced the researchers to accelerate their efforts. Still, Munro estimates it will be at least a year before the vaccine will be ready.



Scientists have determined the 3-D structure of the COVID-19 coronavirus' spike protein (seen in this illustration), which helps the virus enter cells. The work reveals that the protein binds more tightly to proteins on the surface of human cells than the SARS' version of the protein does. Tighter binding may account for the new virus's greater infectivity.

JASON MCLELLAN/UNIV. OF TEXAS AT AUSTIN

"I know the timeline feels long," he says. "I imagine it feels just unacceptable to those folks who are in areas of serious outbreak, but at least we have a way of . . . pushing things forward as fast as possible."

CEPI has calls out for additional vaccine development proposals. On January 31, the organization announced that it would work with CureVac AG, based in Tübingen, Germany, to develop another mRNA vaccine targeting the novel coronavirus.

Beating vaccines to the punch

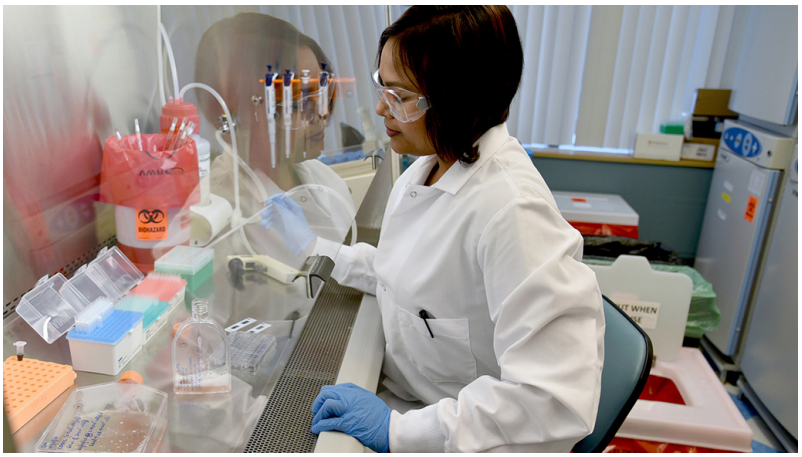
Vaccines help keep people from getting infected with disease-causing organisms but may not help once someone is already infected. But a shortcut to getting protection — a shot of the protective antibodies themselves — may both prevent infections and treat them.

People who have recovered from infections retain antibodies in their blood against the virus or bacteria that caused the illness, often for years or decades. Such antibodies may give some protection when the person encounters a

similar infectious organism later on. But, crucially, these antibodies can also protect others. And quickly.

It can take weeks to months for vaccines to prod the immune system into making protective levels of antibodies, says Christos Kyratsous, vice president of infectious disease research and viral vector technologies at Regeneron Pharmaceuticals. Ebola vaccines, for example, take at least a week to stimulate antibody production, but shots of "antibodies offer immediate protection," Kyratsous says. (Regeneron Pharmaceuticals, headquartered in Tarrytown, N.Y., is a major financial supporter of Society for Science & the Public, which publishes *Science News*.)

In studies conducted by other researchers, blood serum containing protective antibodies taken from people who had recovered from Ebola helped infected people recover from the disease. Doctors and scientists in China have already begun using blood plasma from people who have recovered from COVID-19 to treat people who are ill with the disease.



Scientists at Regeneron's infectious disease labs in Tarrytown, N.Y., are working to develop antibodies to combat the new coronavirus in people.

RANI LEVY/REGENERON

But giving people antibodies from survivors doesn't always work. Regeneron and other companies have developed antibodies that can more reliably offer protection. The company is already testing antibodies against Ebola and the MERS virus. Clinical studies and laboratory work with the company's MERS antibodies suggests that they can help protect against infection and treat established infections, Kyratsous says.

The company is now developing antibodies against the new coronavirus. "We have learned a lot of things from the MERS project that we can now apply to the novel coronavirus project," Kyratsous says.

For instance, the team has learned more about which viral proteins and parts of proteins make the best antibody targets. Proteins on the surface of the virus that are needed for infection, such as the spike protein, are generally the best bets, he says.

Regeneron researchers have made SARS-CoV-2 proteins in the lab and injected them into mice that have human versions of antibody-producing genes. These "humanized mice make fully human antibodies," Kyratsous says, and could provide a ready supply. As soon as those antibodies are available, the company hopes to test their efficacy against the virus in the lab. If that works, safety testing in animals and people may start soon.

The team also hopes to work with people who have recovered from COVID-19 to get antibody-producing cells from their blood. But, Kyratsous says, harvesting antibodies from people isn't something that can be easily scaled up.

Still, despite the rapid reaction of these and other scientists, vaccine and antibody protection for most people is still far off.

"In an acute situation, you're not just going to pull a vaccine out of your pocket," NIAID director Fauci said at the Aspen Institute discussion. If the current outbreak proves to be "really bad," the FDA may be able to authorize emergency use of promising vaccines that haven't completed full safety and efficacy testing. But researchers won't know for at least six months whether any of the vaccines in development help against SARS-CoV-2.

Other strategies to fight the new virus, including repurposing existing drugs used against other diseases, including HIV and hepatitis C, are also under way. But there's no clear winner yet among those candidates. For now, people exposed to the virus must rely on their own immune systems and supportive care from doctors and nurses to fight off the disease.

A version of this article appears in the March 14, 2020 issue of *Science News*.

CITATIONS

K. Modjarrad *et al*. Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial. *The Lancet Infectious Diseases*. Published online July 24, 2019. doi: 10.1016/S1473-3099(19)30266-X.

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EXHIBIT 380

Protection Without a Vaccine

By Carl Zimmer

March 9, 2015

Last month, a team of scientists announced what could prove to be an enormous step forward in the fight against H.I.V.

Scientists at Scripps Research Institute said they had developed an artificial antibody that, once in the blood, grabbed hold of the virus and inactivated it. The molecule can eliminate H.I.V. from infected monkeys and protect them from future infections.

But this treatment is not a vaccine, not in any ordinary sense. By delivering synthetic genes into the muscles of the monkeys, the scientists are essentially re-engineering the animals to resist disease. Researchers are testing this novel approach not just against H.I.V., but also Ebola, malaria, influenza and hepatitis.

“The sky’s the limit,” said Michael Farzan, an immunologist at Scripps and lead author of the new study.

Dr. Farzan and other scientists are increasingly hopeful that this technique may be able to provide long-term protection against diseases for which vaccines have failed. The first human trial based on this strategy — called immunoprophylaxis by gene transfer, or I.G.T. — is underway, and several new ones are planned.

“It could revolutionize the way we immunize against public health threats in the future,” said Dr. Gary J. Nabel, the chief scientific officer of Sanofi, a pharmaceutical company that produces a wide range of vaccines.

Whether I.G.T. will succeed is still an open question. Researchers still need to gauge its safety and effectiveness in humans. And the prospect of genetically engineering people to resist infectious diseases may raise concerns among patients.

“The reality is we are touching third rails, and so it’s going to take some explanation,” said Dr. David Baltimore, a Nobel Prize recipient and virologist at Caltech who is testing I.G.T. against a number of diseases.

Conventional vaccines prompt the immune system to learn how to make antibodies by introducing it to weakened or dead pathogens, or even just their molecular fragments. Our immune cells produce a range of antibodies, some of which can fight these infections.

In some cases, these antibodies provide strong defenses. Vaccinations against diseases such as smallpox and measles can lead to almost complete protection.

But against other diseases, conventional vaccines often fail to produce effective antibodies. H.I.V., for example, comes in so many different strains that a vaccine that can protect against one will not work against others.

I.G.T. is altogether different from traditional vaccination. It is instead a form of gene therapy. Scientists isolate the genes that produce powerful antibodies against certain diseases and then synthesize artificial versions. The genes are placed into viruses and injected into human tissue, usually muscle.

The viruses invade human cells with their DNA payloads, and the synthetic gene is incorporated into the recipient’s own DNA. If all goes well, the new genes instruct the cells to begin manufacturing powerful antibodies.

Dr. Michael Farzan, an immunologist at Scripps Research Institute, helped develop an artificial antibody that inactivated H.I.V. in monkeys. Benjamin Rusnak for The New York Times

The idea for I.G.T. emerged during the fight against H.I.V. In a few people, it turned out, some antibodies against H.I.V. turn out to be extremely potent. So-called broadly neutralizing antibodies can latch onto many different strains of the virus and keep them from infecting new cells.

Dr. Philip R. Johnson, chief scientific officer of The Children's Hospital of Philadelphia and a virologist at the University of Pennsylvania, had an idea: Why not try to give broadly neutralizing antibodies to everybody?

At the time, Dr. Johnson and other researchers were experimenting with gene therapy for disorders like hemophilia. Researchers had figured out how to load genes into viruses and persuade them to invade cells, and it occurred to Dr. Johnson that he might be able to use this strategy to introduce the gene for a powerful antibody into a patient's cells.

After the cells began producing antibodies, the patient in effect would be "vaccinated" against a disease.

The idea represented a radical new direction for gene therapy. Until then, researchers had focused on curing genetic disorders by providing working versions of defective genes. I.G.T., on the other hand, would protect healthy people from infectious diseases.

And there was no guarantee that it would succeed. For one thing, the best virus Dr. Johnson had for delivering genes worked only to invade muscle cells — which normally would never make antibodies.

In 2009, Dr. Johnson and his colleagues announced that the approach worked after all. In their experiment, they sought to protect monkeys from S.I.V., a primate version of H.I.V. To do so, they used viruses to deliver powerful genes to the monkeys' muscles.

The muscle cells produced S.I.V. antibodies, as Dr. Johnson and his colleagues had hoped. Then they infected the monkeys with S.I.V. The monkeys produced enough antibodies in their muscles to protect them from S.I.V. infections, the scientists found. Without the I.G.T. procedure, monkeys dosed with the virus died.

Dr. Johnson's study persuaded Dr. Farzan that I.G.T. has great promise. "I started drinking the Kool-Aid," he said. Dr. Farzan and his colleagues have been modifying H.I.V. antibodies to develop more potent defenses against the virus.

Meanwhile, in 2011, Dr. Baltimore and his colleagues showed that antibodies delivered into cells with viruses could protect mice against injections of H.I.V., suggesting that I.G.T. could protect people against H.I.V. in contaminated needles.

But most H.I.V. infections occur through sex. So Dr. Baltimore and his colleagues also infected female mice with H.I.V. through their vaginal membranes. Last year, they reported that the technique also protected mice from infection in this way.

"We're going around the immune system, rather than trying to stimulate the immune system," Dr. Baltimore said. "So what we're doing is pretty fundamentally different from vaccination, although the end result is pretty similar."

Gary W. Ketner, a microbiologist at the Johns Hopkins Bloomberg School of Public Health, was intrigued by Dr. Baltimore's results and wondered if I.G.T. could be marshaled against another major disease that has eluded vaccines: malaria.

Dr. Ketner, Dr. Baltimore and their colleagues found a potent antibody against malaria and used a virus to deliver the gene for making it into mice. Last August, they reported that when malaria-laden mosquitoes bit the mice, up to 80 percent of the treated animals were protected.

Dr. Philip R. Johnson, chief scientific officer of The Children's Hospital of Philadelphia and a virologist at the University of Pennsylvania, developed an approach of giving neutralizing antibodies to healthy people. Jessica Kourkounis for The New York Times

"It is encouraging," Dr. Ketner said. "It's good for a first shot of an unproven method, but it should be better." Now Dr. Ketner is searching for better antibodies that provide more protection in a smaller dose.

These experiments suggest that antibodies created by I.G.T. could help against diseases that have resisted vaccines for decades. Other studies suggest that I.G.T. might also help against sudden outbreaks in the future.

Dr. James M. Wilson, a pathologist at the University of Pennsylvania, and his colleagues have investigated using gene therapy to treat cystic fibrosis by delivering genes into the cells lining patients' airways. It occurred to him that many fast-spreading viruses, such as influenza and SARS, also attack the same cells.

In 2013, Dr. Wilson and his colleagues reported that viruses carrying antibody genes into airway cells can enable mice and ferrets to fight off a wide range of flu strains. Since then, he and his colleagues have tested I.G.T. against other viruses causing deadly outbreaks — including Ebola.

Dr. Wilson and his colleagues teamed with Mapp Biopharmaceutical, a company that has developed an antibody against Ebola called ZMapp. The scientists have synthesized a gene for the ZMapp antibody and have delivered the gene into mouse muscles. The experiments are only in their early stages, but "we have encouraging data," Dr. Wilson said.

For Dr. Johnson, the growing interesting in I.G.T. is gratifying. "It's catching on, but it's certainly not mainstream," he said. That seems likely to change, and soon.

Last February, Dr. Johnson began the first clinical trial of I.G.T. in humans. His team has placed H.I.V. antibody genes into the muscles of volunteers to see if the treatment is safe. The researchers expect to finish gathering the results this spring. "We're optimistic. We're hopeful," Dr. Johnson said.

Dr. Baltimore is collaborating with the National Institutes of Health to start a similar trial of an I.G.T.-engineered virus against H.I.V. Dr. Wilson is preparing to test I.G.T. against the flu later this year.

There is no guarantee that the successes in the animal trials can be replicated in humans. "Humans are not just big mice," said Dr. Ronald G. Crystal, chairman of genetic medicine at Weill Cornell Medical College.

Human immune systems may attack the artificial antibodies or the viruses delivering them, destroying their protection. Or muscle cells might make too many antibodies, because they do not have the built-in regulation that immune cells do.

Dr. Farzan and other researchers are investigating molecular switches that can turn off the production of antibodies, or just adjust their dose. "If we really want to see this blossom, we need regulatory 'off' switches," he said.

Despite the lingering concerns about I.G.T., Dr. Nabel says he remains optimistic. "There are safety concerns that have to be addressed, but there are logical ways to approach them," he said.

Bioethicists do not foresee major ethical hurdles to I.G.T., because it is based on gene therapy, which has been developed for more than 30 years. "It doesn't strike me as a radical departure," said Jonathan Kimmelman, an associate professor at McGill University.

Still, Dr. Baltimore says that he envisions that some people might be leery of a vaccination strategy that means altering their own DNA, even if it prevents a potentially fatal disease.

"But my feeling, as a basic scientist, is that it's our responsibility to take things into the clinic that we feel will make a difference," he said.

EXHIBIT 381

frontiers in Immunology

REVIEW
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Nanoparticle Vaccines Against Infectious Diseases

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Due to emergence of new variants of pathogenic micro-organisms the treatment and immunization of infectious diseases have become a great challenge in the past few years. In the context of vaccine development remarkable efforts have been made to develop new vaccines and also to improve the efficacy of existing vaccines against specific diseases. To date, some vaccines are developed from protein subunits or killed pathogens, whilst several vaccines are based on live-attenuated organisms, which carry the risk of regaining their pathogenicity under certain immunocompromised conditions. To avoid this, the development of risk-free effective vaccines in conjunction with adequate delivery systems are considered as an imperative need to obtain desired humoral and cell-mediated immunity against infectious diseases. In the last several years, the use of nanoparticle-based vaccines has received a great attention to improve vaccine efficacy, immunization strategies, and targeted delivery to achieve desired immune responses at the cellular level. To improve vaccine efficacy, these nanocarriers should protect the antigens from premature proteolytic degradation, facilitate antigen uptake and processing by antigen presenting cells, control release, and should be safe for human use. Nanocarriers composed of lipids, proteins, metals or polymers have already been used to attain some of these attributes. In this context, several physico-chemical properties of nanoparticles play an important role in the determination of vaccine efficacy. This review article focuses on the applications of nanocarrier-based vaccine formulations and the strategies used for the functionalization of nanoparticles to accomplish efficient delivery of vaccines in order to induce desired host immunity against infectious diseases.

Keywords: nanoparticles, vaccine development, human diseases, targeted vaccine delivery, antigens

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INTRODUCTION

In twenty-first Century, infectious diseases have emerged as a serious threat to the health of millions of people across the globe (1). According to the World Health Organization (WHO) report for 2016, ∼3.2 million deaths have occurred due to lower respiratory infections and 1.4 million from tuberculosis alone worldwide (2). Over the past few decades, many new infectious diseases have emerged and few old diseases re-emerged, which were once considered to be no longer a threat to the human being (3–5). Collectively, these diseases account for millions of deaths that cause enormous impact on the global socio-economical and health-care sectors. The major challenges to combat such diseases are that for many of them, there are no effective drugs available.

One of the plausible approaches could be based on the application of nanocarrier based vaccination (6). However, there are still no effective vaccines available against some of the most prevalent diseases including immune deficiency syndrome (AIDS) and tuberculosis. This underlines an urgent need for the development of desired vaccines against these diseases. Some of the important aspects of any optimal vaccine includes (i) safety, (ii) stability, and (iii) the ability to elicit durable and adequate immune response with a minimum number of doses (7–9). Presently, different generation vaccines such as attenuated or killed whole organisms (*first generation*), subunit (*second generation*) and RNA or DNA vaccines (*third generation*) are used to elicit protective immunity against diseases (10–12). Despite several advantages of RNA or DNA vaccines such as minimal risk of infection, ability to elicit immune response against specific pathogen and cost effective (13); there are a number of challenges associated with the efficient delivery of these vaccine molecules to the target sites and the requirement of the prime-boost vaccination regimens with other immunogenic agents. These includes premature degradation of molecules and the inability to translate into a functional immunogen (14). Similarly, protein based vaccines are used successfully against several infectious diseases such as *Haemophilus influenza* type b, diphtheria, tetanus, acellular pertusis, meningococcus and pneumococcus (15), however they require an adjuvant to potentiate their immunogenicity, and also encounter early degradation after exposure to hostile milieu. Introduced recombinant protein-based vaccines (e.g., recombinant hemagglutinin vaccine for influenza) further enhance the immunity toward infection indicating the applicability of the recombinant technology for the vaccine production (16). To overcome these hurdles, an efficient vaccine delivery system is required which not only delivers the vaccine molecules to the target site to evoke enduring immune responses but also has minimal side effects and requires less doses. Moreover, there is an increasing need to develop new generation composite vaccine molecules that will act as immunogen as well as an adjuvant. Nanotechnology based formulations offer numerous advantages for the development of new generation vaccines. Nanocarrier based delivery system can protect the vaccines from premature degradation, improve stability, has good adjuvant properties, and also assists in targeted delivery of an immunogen to the antigen presenting cells (APCs). There are several mechanisms by which vaccines can be delivered to the specific sites using nanocarriers. Vaccine antigens can be encapsulated within the nanocarriers or decorated on their surface (**Figure 1**). Encapsulation within the nanoparticles (NPs) can protect the antigen from premature protease degradation and elicit sustainable release, whereas the surface adsorption facilitates their interaction with cognate surface receptors such as toll like receptors (TLRs) of APCs (17). Nanocarrier based delivery systems provide a suitable route of administration of vaccine molecules and enhance cellular uptake thereby resulting in robust innate, humoral, cellular as well as mucosal immune responses when compared with unconjugated antigens. This review mainly focuses on the potential use of nano delivery systems as novel vaccine strategies for the induction of innate as well as adaptive immune responses against infectious diseases.

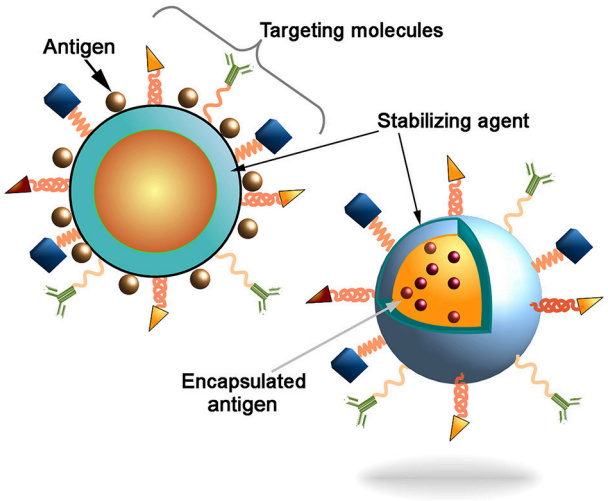

FIGURE 1 | Schematic representation of the nanocarriers. Antigen can be conjugated to the nanoparticles surface or incapsulated into core of the particles. Decoration of the nanoparticles surface with targeting molecules (e.g., antibodies, Fab-fragments, peptides, etc) could further increase the delivery of particles into the antigen presenting cells (APCs) to induce innate and adaptive immune responses.

KEY CELLULAR COMPONENTS OF THE IMMUNE SYSTEM

The immune system is composed of a collection of mobile cells that traffic throughout the body as well as reside at the site of entry (i.e., skin, respiratory, gastrointestinal, and genital tracts) in search of invading pathogens. These cells belong to two major types of innate and adaptive immune system. The innate immune cells like macrophages and neutrophils rapidly respond to the pathogens by recognizing pathogen surface moieties, phagocytosis, and the elimination of pathogens through activation of different antibacterial effector functions. Similarly, two major components of the adaptive immunity i.e., T and B-cells are important for the generation of cell mediated and humoral immune responses, respectively. T cells including $CD4^+$ helper T cells secrete different cytokines to modulate the functions of B cells, whereas $CD8^+$ T cells recognize and destroy virally infected cells. Antibodies produced by the B cells can further neutralize the invading microbes or clear infected cell or opsonized pathogens through cell-mediated systems. APCs, in particular dendritic cells (DCs) and macrophages, migrate through the body to sample, process and present the antigens to T-cells to activate cellular immune responses. These cells express various surface receptors to recognize cognate ligands and danger signals to trigger activation of different signaling pathways that eventually lead to the activation of T-cells (18). After sampling the antigens, DCs migrate from the peripheral tissues into the draining lymph nodes to activate naive T-cells (19), whereas macrophages after ingestion of antigens increase their lysosomal degradative machinery to enhance the antigen presentation to activate helper T cells.

TYPES OF NANO-IMMUNO ACTIVATORS

Some NPs are themselves able to stimulate different immune cells to boost the host immunity. The size, shape and surface chemistry of NPs (described below in more detail) are important factors that determine their potential to activate immune responses. In general, NPs are able to stimulate immune reactions by increasing the synthesis of defense genes and inflammatory reactions (20). Various types of NPs like gold, carbon, dendrimers, polymers and liposomes have the capability to induce cytokine and antibody responses (21–26). This was observed in the case of administration of empty PEGylated liposomes, which were able to elicit IgM response in an *in-vivo* model. (27, 28). Besides their potential to deliver various immune stimulators to the specific sites as well as into the deep tissues where vaccine molecules alone may not able to reach, these NPs have also been exploited as adjuvants to augment immunogenicity of vaccine candidates. Nano-immuno stimulators are the nano scale (20–100 nm) vaccine particles that can improve the vaccine efficacy *in vivo* better than bulk molecules (20, 29). Some of the known nano-immuno stimulators that have been used for this specific purpose are inorganic NPs (iron and silica) (30, 31), polymeric NPs (chitosan, PLGA, PVPONAlk, γ-PGA) (32–37), liposomes (cholesterol and lipids) (33, 38) and virus like particles (VLPs) (39, 40). Different types of NPs used to deliver antigens to give protection against different diseases have been listed in **Table 1**.

Inorganic NPs

Some biocompatible inorganic NPs such as gold, carbon and silica have been exploited in the vaccine delivery studies (50, 79–81). These NPs can be synthesized in various shapes, size and surface modified forms. Some of the viral antigens were successfully delivered using inorganic NPs as carriers. This caused increase in antigen stability by protecting them from premature degradation by proteolytic enzymes. Delivery of viral and bacterial antigens using gold NPs was also found to induce quite robust host immune responses against influenza, immunodeficiency virus, foot and mouth, and tuberculosis diseases in mice (51, 52, 82, 83). Encapsulation of plasmid DNA that encode mycobacterial hsp65 antigen in gold NPs exhibited significant reduction in the *Mycobacterium tuberculosis,* causative agent of human tuberculosis, burden in infected mice (52, 82). Few studies have used hollow mesosporous silica, nanotube and spherical forms of carbon NPs as adjuvants to improve the immunogenicity and delivery of protein and peptide antigens against viral infections (79, 83, 84). Silica based NPs contain abundant silanol groups that can be utilized to introduce specific functional groups on their surface to gain access for vaccine molecules into target cells (84–86). The major advantages of inorganic NPs include low production cost, reproducibility and safety in application.

Polymeric NPs

In recent years, polymeric NPs have received great attention for their applications in the delivery of a number of vaccines. This is primarily due to their ease in preparation, biodegradability, biocompatibility, reduced cytotoxicity, and the possibility to fine-tune surface properties as needed (87). Moreover, it is relatively easy to control the rate of vaccine release by altering the composition or ratio of co-polymers during the NP synthesis process (87). The most commonly used polymeric NPs for vaccine delivery are poly (lactic-*co*-glycolic acid; PLGA) or poly (lactic acid; PLA). PLGA NPs have already been tried in the delivery of a broad range of antigens, including hydrophobic antigens (34, 35), hepatitis-B virus antigens (54), *Bacillus anthracis* (41), tetanus toxoid (35), and ovalbumin (88). The use of PLGA conjugated antigens exhibited strong immuno-stimulatory property by inducing cytokine and nitric oxide production against mycobacteria infection (89). In addition to synthetic polymers, some natural biopolymers such as alginate, pullans, inulins, and chitosan have been used as adjuvants (90–93). Inulin, a known activator of the complement cascade (94), conferred better protection against hepatitis B and influenza viruses (92, 93). Similarly, chitosan NPs were demonstrated as nanocarrier molecules for HBV antigens (55), DNA vaccine (56), and Newcastle disease vaccine (42). The delivery of PLGA and chitosan NP conjugated vaccine molecules enhanced the immune responses at the mucosal site (95, 96). Our recent study also showed that delivery of *M. tuberculosis* lipids using biocompatible chitosan NPs was able to induce significant humoral as well as cellular immune responses when compared to lipids alone in mice (43). We also found that intraperitoneal administration of these conjugates showed better activation of splenic T-cells. Another study by de Titta et al. has shown that intradermal administration of CpG conjugated polymeric NPs increased dendritic cell activation by several fold, exhibited comparable vaccine efficacy at ∼400 times lower dose, and also caused enduring cellular immunity in comparison to free CpG (97). These desired properties along with already known reduced toxicity and biocompatibility under both *in vitro* and *in vivo* conditions make polymeric NPs plausible candidates for further preclinical pharmacokinetics and therapeutic applications (98).

Liposomes

In addition to polymeric NPs, liposomes are the second most widely explored vaccine and drug delivery vehicle in the nanomedicine field. The synthesis of liposomes is a spontaneous process, where hydration of lipids enables the lipid bilayer formation around an aqueous core (99). So far, different types of liposomes, including unilamellar or multilamellar vesicles composed of biodegradable phospholipids (e.g., phosphatidylserine, phosphatidylcholin and cholesterol) were included in the vaccine studies (100). Liposomes deliver vaccines by fusion with the target cell membrane (101).The structurally flexible and versatile liposomes are able to encapsulate both hydrophilic and hydrophobic substances. The hydrophilic molecules can be incorporated into the aqueous core, while hydrophobic molecules are encased within the phospholipid bilayer. Earlier reports have shown that delivery of antigenic proteins entrapped in multilamellar lipid vesicles elicit strong T and B-cell responses (102). Similarly antigenic peptides conjugated to phosphatidylserine (PS)-liposomes were readily

TABLE 1 | List of antigens delivered by using different nanocarriers for the treatment of different diseases.

Antigen	Nanocarrier used	Disease	References
AGAINST BACTERIAL INFECTION			
Antigenic protein	Poly(D,L-lactic-co-glycolic acid) nanospheres	Anthrax	(41)
DNA encoding T cell epitopes of Esat-6 and FL	Chitosan Nanoparticle	Tuberculosis	(42)
Mycobacterium lipids	Chitosan Nanoparticle	Tuberculosis	(43)
Polysaccharides	Liposomes	Pneumonia	(44)
Bacterial toxic and parasitic protein	Liposomes	Cholera and Malaria	(45)
Fusion protein	Liposomes	*Helicobacter pylori* infection	(46)
Antigenic protein	Nanoemulsion	Cystic fibrosis	(47)
Antigenic protein	Nanoemulsion	Anthrax	(48)
Mycobacterium fusion protein	Liposome	Tuberculosis	(49)
AGAINST VIRAL INFECTION			
Antigenic protein	Chitosan Nanoparticles	Hepatitis B	(33)
Viral protein	Gold Nanoparticles	Foot and mouth disease	(50)
Membrane protein	Gold Nanoparticles	Influenza	(51)
Viral plasmid DNA	Gold Nanoparticles	HIV	(52)
Tetanus toxoid	Poly(D,L-lactic-co-glycolic acid) nanospheres	Tetanus	(53)
Hepatitis B surface antigen	Poly(D,L-lactic-co-glycolic acid) nanospheres	Hepatitis B	(54)
Hepatitis B surface antigen	Alginate coated chitosan Nanoparticle	Hepatitis B	(55)
Live virus vaccine	Chitosan Nanoparticles	Newcastle disease	(56)
Capsid protein	VLPs	Norwalk virus infection	(57)
Capsid protein	VLPs	Norwalk virus infection	(58)
Influenza virus structural protein	VLPs	Influenza	(59–64)
Nucleocapsid protein	VLPs	Hepatitis	(65)
Fusion protein	VLPs	Human papilloma virus	(39, 40, 66–68)
Multiple proteins	VLPs	Rotavirus	(69, 70)
Virus proteins	VLPs	Blue tongue virus	(71)
Enveloped single protein	VLPs	HIV	(72–75)
Viral protein	Polypeptide Nanoparticles	Corona virus for Severe acute respiratory syndrome (SARS)	(76)
AGAINST PARASITIC INFECTION			
Merozoite surface protein	Iron oxide Nanoparticles	Malaria	(30)
Epitope of *Plasmodium berghei* circumsporozoite protein.	Polypeptide Nanoparticles	Rhodent mamarial parasitic infection	(77)
Surface protein from *Eimeria falciformis* sporozoites	ISCOMs	Diarrhea	(78)

internalized by APCs to potentiate T-helper cell mediated immune responses (103) and delivery of heat shock protein encoding vaccine DNA using liposomes elicited strong protective immunity against fungal infection (104). Because of their foreseen applications, several liposome based vaccine nano-formulations have been approved for clinical trials against intracellular pathogens, including viruses and *M. tuberculosis* (105). One such study already demonstrated the potency of liposomal aerosol carriers in the generation of protective immunity against *M. tuberculosis* infection (106, 107). Other studies have tried a combination of dimethyl dioctadecyl ammonium (DDA) lipid based liposomes and various immunomodulators to enhance immunity against influenza, chlamydia, erythrocytic-stage malaria, and tuberculosis infections (108–112). In the context of DNA vaccines, lipid-DNA complexes have been successfully delivered to the lungs of monkeys (101).

VLPs (Virus Like Particles)

There are several reports that adequately proved applications of VLPs as a vaccine carrier, and also their ability to stimulate the host immune responses (113–115). VLPs are composed of self-assembled viral membrane that forms a monomeric complex displaying a high density of epitopes (115, 116). Interestingly, VLPs can also be engineered to express additional proteins either by fusion of proteins with the particles or by endogenous expression of multiple antigens (113, 117). It is also possible to chemically couple non-protein antigens and small organic molecules onto the viral surface to produce bioconjugates with VLPs (118, 119). Due to these distinct features, VLPs can provide protection not only against virus, but also against heterologous antigens (116). A specific immune response was successfully generated after the delivery of an antigen using virus capsid protein SV40 in mammalian cells (120). VLPs were also found to increase the immunogenicity of weak antigens. For example

Salmonella typhi membrane antigen, influenza A M2 protein and H1V1 Nef gonadotropin releasing hormone (GnRH) assembled VLPs produced strong antigen specific humoral as well as cellular immune responses (121, 122). It is presumed that the use of VLP based nanoformulations could enable the antigens to achieve conformations resembling to native antigen structure, thus it may result in better stimulation of the host immune response (122).

Dendrimers

Dendrimers are three dimensional, mono-dispersed and hyperbranched nano structures that are made up of a mixture of amines and amides. Few studies have explored the application of dendrimers in the delivery of different antigenic molecules. The most commonly used dendrimers for vaccine delivery are polypropyleneimine (PPI) and polyamido amine (PAMAM) dendrimers. A single dose of dendrimer encapsulated multiple antigens was found to produce strong antibody and T-cell responses against Ebola virus, H1N1 influenza, and *Toxoplasma gondii* (123). This generation of robust immune response was found to be due to efficient uptake of dendrimers by the host cells. Similarly a significant increase in the vaccine efficacy of HIV transactivator of transcription (TAT) based DNA vaccine was observed due to enhanced cellular uptake of PMAM dendrimer (124). Hence, the possibility to tailor the dendrimers to attain certain biological and physico-chemical properties, and also the feasibility to conjugate several ligands to the single molecule have made dendrimers promising candidates for the development of new generation vaccines with enhanced immunogenic properties.

DELIVERY OF IMMUNE STIMULATORS USING NANOCARRIERS

Cytokines

Cytokines are known as important signaling molecules secreted by different cells in response to external stimuli. Some of the cytokines are able to activate immune cells to generate protective immunity against several diseases. However, cytokines are mostly susceptible to early degradation that subdue their participation in the generation of host immunity. Moreover, uncontrolled release of cytokines as immune responders may sometimes lead to harmful side effects (125). To overcome these limitations, several studies have attempted to synthesize engineered nanocarriers to achieve effective and controlled delivery of cytokines to the target sites. This approach was found to reduce their toxicity, improve circulation time and antigen specific T-cell responses in comparison to free cytokines (126, 127). Incorporation of granulocyte macrophage colony stimulating factor (GM-CSF) and interferon alpha (IFN-α) into nano-carriers exhibited great application in cancer therapy (128, 129). Nano-carrier conjugated cytokines also showed great potential in the treatment of infectious diseases. For example, IL-12 encapsulated microspheres induced strong protective immunity against tuberculosis (130). This effect was due to production of high antibody titers as a result of sustained and controlled release of IL-12 from the microspheres in immunized mice (130).

Toll Like Receptor Agonists

Like cytokines, several toll-like receptor (TLR) agonists were also explored as immune activators to augment immune surveillance mechanisms. Different immune effector cells such as macrophages, B-cells and DCs express different types of TLRs, which are known to interact with specific pathogen associated molecular patterns (PAMPs). These specific interactions eventually initiate downstream signaling cascades to ensure the elimination or generation of immunity against pathogens (131, 132). Conjugation of TLR specific agonists on nanocarriers helps to target the molecules to specific immune cells and therefore reduce the possibility of systemic biodistribution. One such study has shown that conjugation of TLR-7/8 agonist on nano polymers caused efficient internalization by APCs and also prolonged the T cell responses (133). Administration of NPs loaded with vaccine peptide antigen and TLR-7 and 9 ligands were also found to induce strong memory and effector $CD8^+$ T-cell response (134). Another study has shown that conjugation of TLR-8 agonist to a polymer nanocarrier increased activation and maturation of naive DCs due to selective endocytosis and prolonged release of an immunogen by the nanocarrier inside DCs (135). Moreover, intradermal injection of CpG and antigen encapsulated polymeric NPs were rapidly drained into the lymph nodes to activate DCs (97). These studies indicate that NPs can be used as a tool to appropriately target presentation of antigens to T and B-cell rich lymphoid organs.

Nucleic Acids

The genetic molecules such as DNA, plasmids and RNA can also act as immuno-stimulants. Due to these characteristics, in addition to less risk to cause disease particularly in immunocompromised individuals, these genetic materials are considered as promising candidates for the development of next generation vaccines. After administration, the plasmid vector translocates to the nucleus to initiate transcription of recombinant genes using the host cellular machinery. A recombinant DNA segment encoding HspX-PPE44-esxV fusion antigen of *M. tuberculosis* showed great potential as a new tuberculosis DNA vaccine candidate (136). A similar type of study has been conducted in the past where the vaccination of DNA or RNA constructs expressing mycobacterium antigens were capable of inducing humoral as well as cellular immune responses (137). Likewise, plasmids harboring genes encoding for viral antigen have been encapsulated into alginate nanocarriers and targeted against viral infections (138).

IMPORTANCE OF PHYSICOCHEMICAL PROPERTIES IN DESIGNING NANO-IMMUNO FORMULATIONS

In order to improve their delivery and vaccine characteristics, different approaches have been practiced to conjugate vaccine molecules to different nanocarriers. Vaccine molecules can be surface conjugated, encapsulated or surface adsorbed with the nanocarriers. Antigen adsorption on the nanocarrier is simply based on the presence of a charge or hydrophobic interactions

between NPs and the candidate molecule (139, 140). This type of interaction is usually non-covalent, which may lead to rapid dissociation of antigens from nanocarriers depending upon the external milieu such as pH, ionic strength, temperature, and the antigen hydrophobicity. On the other hand, encapsulation and chemical conjugation of antigen to nanocarriers is more stable due to strong interactions and chemical bond formation between the target molecule and the nanocarrier. Further, antigens can also be encapsulated into nanocarriers by simple mixing reaction during the synthesis. In this case, the antigens are released only after partial or complete dissociation of the nanocarrier (141). These processes have already been used with silica and gold NPs (142). Similarly, chitosan and dextran sulfate NPs were used for the preparation of cationic and anionic antigenic formulations. Some viral antigens are known to bind to both positive as well as negative charged NPs through immobilization process and hydrogen bonds (143). The immobilization process depends on the charge, pH, ratio of NPs and antigens, and the protein partition coefficient between the solution and the colloid (143). Several antigens were successfully delivered to the target sites by chemical conjugation, adsorption and encapsulation to soft nanocarriers like VLPs, liposomes and immune stimulating complexes (ISCOM) (144–147). ISCOMs are a class of adjuvant formulations that consist of saponins, cholesterol and phospholipids in specific ratios. Antigens can be formulated into ISCOMs directly (148) or after the surface modification (149, 150). Since ISCOM particles are negatively charged, direct conjugation of most of the soluble proteins is a limiting factor. Nanocarriers can augment immunogenicity of a molecule. For example, influenza antigen H1N1 conjugated chitosan NPs and *Yersinia pestis* F1-antigen coated gold NPs (AuNPs) produced higher levels of antibody and cytokine responses in comparison to mice administered with unconjugated antigens (151).This was found to be due to stabilization and increased immunogenicity of vaccine antigens due to conjugation with NPs.

Another important aspect in the development of nano-immuno formulations is that they improve antigen delivery and presentation (152). In this context, NP shape, size and surface charge are key factors that affect NP circulation, biodistribution, bioavailability and specificity by crossing biological barriers. Besides these factors, particle geometry such as surface to volume ratio plays an important role in the determination of immunogen release and degradation kinetics (153, 154). Here, the importance of different physicochemical parameters such as size, shape, surface area, porosity, hydrophobicity, hydrophilicity and crystallinity in the interaction between NPs and the target cell is discussed.

Size

The size of NPs determines the mode of cellular uptake and specificity (155, 156). PLGA NPs of large size (1, 7 and 17 μm) showed reduced internalization rate in comparison to smaller NPs (300 nm) (157). The size of NPs also determines the cellular specificity and migration. Smaller NPs (20–200 nm) were readily endocytosed by the resident DCs, whereas larger size (500–2,000 nm) NPs were effectively taken up by the migratory DCs (158). NPs of less than 200 nm size were drained into the lymph nodes (159), while particles up to 20 nm range were suitably transported to the APCs (152, 160). Notably, NP curvature also affects the cellular interaction and phagocytosis rate (161). NPs of 150 nm diameter and 450 nm height showed more cellular uptake as compared to the particles having 1,200 × 200 nm size. Of note the size of NPs was also found to influence the activation of signaling pathways. A study has demonstrated that smaller NPs are able to alter the cell signaling processes more efficaciously than the large NPs (31).

Surface Charge

Vaccine loaded NPs can also be targeted to specific sites by modifying the NP surface charge. Delivery of such NPs at appropriate sites elicit strong immune responses against antigens. NP surface charge is responsible for the interaction with congnate surface molecules present on the target cells. This was exemplified from the observation that cationic polysterene NPs were efficiently internalized by the APCs in comparison to neutral surface charged NPs. This may be due to electrostatic interactions between the cationic NPs with anionic cell membranes (162, 163). Interestingly, pulmonary instillation of cationic and anionic NPs showed similar endocytosis rate in macrophages and draining lymph nodes, however cationic formulations showed more expression of *Ccl2* and *Cxc10* chemokines that caused more recruitment and maturation of CD11b DCs in comparison to anionic NPs in the lung (125, 156). Similarly, neutral silica-silane shell polymer NPs were less effective in the activation of innate immune cells (128). These studies clearly indicate appropriate surface modifications of NPs may help to generate stronger immunological responses against specific infection.

Shape

Beside size and surface charge, NP shape is also a critical determinant in the cellular interaction, intracellular trafficking and the rate of antigen release inside the host cells (79, 141). Spherical gold NPs were actively internalized by bone marrow derived dendritic cells in comparison to rod shaped particles of similar dimensions (33, 34), and that spherical NPs were able to induce strong immune response than cube or rod shaped NPs (164). Another study reported that worm-like particles were impaired in phagocytosis as compared to spherical NPs (151). These distinctions were ascribed to the differences in contact area between NPs and the target cell membrane. The shape of NPs also determines the localization of NPs inside the host cells. This was demonstrated by the fact that although nano rods and nano sheets were internalized via clathrin mediated endocytosis, nano rods were particularly delivered to the nucleus while nano-sheet were retained in the cytoplasm (146, 147, 155). This is an important aspect in the context of improving antigen processing and presentation to T-cells. It is well established that enhanced antigen processing and presentation can be achieved if the candidate molecules are delivered to the lysosomal compartment of the cells.

Hydrophobicity

Hydrophobicity of NPs plays a significant role in the interaction with soluble proteins and immune cells through recognition of hydrophobic moieties (165). Previous studies have shown that

hydrophobic polymeric NPs are strong inducers of cytokines and co-stimulatory molecules than hydrophilic polymeric NPs (53, 105, 166). Exposure to hydrophobic NPs showed enhanced activation of DCs by inducing the expression of CD86 co-stimulatory molecules when compared with hydrophilic ones. Similar observations were reported in other innate immune cells, in which hydrophobic NPs were able to activate these cells by up-regulating the expression of proinflammatory cytokine encoding genes (102), and also facilitated opsonization process by increasing the adsorption of immunoglobulins on the cell surfaces (103). However, other studies have reported that polyethylene glycol coating (PEGylation) reduced the interaction of NPs with immune receptors (50, 80). This property is considered useful in the prevention of non-specific adsorption of proteins on NPs and thereby prevent their up-take by APCs (50). Such non-specific adsorption of proteins and their uptake by phagocytic cells can also be preventing by the incorporation of an alkyl linker between the PEG and thiol moieties on NPs (80).

Surface Modification

Surface modification of NPs alters ligand specificity and interaction with APCs (160). Conjugation of CD47 molecules on the surface of NPs modulated the down-stream signaling cascades and also reduced NP internalization by phagocytic cells (131). Functionalization of NPs with TLR-7, TLR-8, and TLR-9 agonists increased cytokine production and the expression of immunoregulatory genes (132–134). Similarly, conjugation of poly (methyl vinyl ether-co-maleic anhydride; PVMA), TLR2, and TLR4 agonists, and galactose polymer to NPs were shown to activate the complement pathway as a result of stable binding to C3b complement factor (139, 142). Further, lipoprotein-like NPs showed LPS scavenging activity, thereby resulting in the inhibition of TLR-4 dependent inflammatory responses (140). Overall, these studies strongly demonstrated that tuning of physico-chemical properties of NPs could be used as a fundamental tool to target vaccine molecules to specific sites to induce desired immune responses.

IMPLICATIONS OF THE NANOCARRIERS IN THE VACCINE DEVELOPMENT

Emerging studies have proved that nanocarriers can be useful mediators in the development of vaccines against various diseases. In this context, it is important to develop NP formulations that can deliver immunogens to APCs especially DCs to induce effective antigen-specific T-cell responses (**Figure 2**). Several nanocarriers have been shown to specifically activate DCs to effectuate anti-tumor or anti-viral immune responses (167–170). Zhu et al. proposed that nano-TiO_2 and Fe_3O_4-TiO_2 particles could function as a useful vector to promote vaccine delivery in immune cells (168). Co-incubation of nano-TiO_2 and Fe_3O_4-TiO_2 with DCs resulted in an increased production of TNF-α, and also upregulated the expression of CD80, CD86 and MHC class II molecules through the NF-κB signaling pathway (163). In this way, immunization efficacy of various NP formulations such as erythrocyte membrane-enveloped poly(D,L-lactide-co-glycolide) (PLGA) NPs for antigenic peptide (hgp10025-33) and TLR-4 agonist, VLPs expressing RSV glycoproteins, chitosan-coated EphrinA1-PE38/GM-CSF, and several others have been improved (171–177). NPs can also control cell polarization and differentiation. Branched polyethylenimine-superparamagnetic iron oxide NPs (bPEI-SPIONs) promoted Th1 polarization of DCs (178). Another study by Sehgal et al. showed that NPs can also be used to target subsets of particular immune cells. They have shown that simultaneous targeting of DC subsets (i.e., DC-SIGN+ and BDCA3+DC) by NPs synergistically stimulated the activation of T cell-mediated immunity when compared with targeting of each DC subset separately (170).

Preclinical studies by different research groups have successfully demonstrated the efficacy of NP based vaccines in the induction of specific immune responses against tuberculosis (42, 179–182). Feng et al. developed a NP-based recombinant DNA vaccine that consists of Esat-6 and fms-like tyrosine kinase 3 ligand enveloped with chitosan NPs (42). Intramuscular prime vaccination followed by nasal boost of this recombinant DNA vaccine remarkably enhanced T cell responses in *Mycobacterium tuberculosis* challenged mice (42). Another study has shown that pulmonary administration of *M. tuberculosis* Ag85B antigen and CpG adjuvant conjugated polypropylene sulfide NPs (NP-Ag85B) induced *M. tuberculosis* specific polyfunctional Th1 responses and also reduced the lung bacterial burden (183).

TARGETED DELIVERY OF NANOPARTICLES CAN ACTIVATE INNATE AND ADAPTIVE IMMUNE RESPONSES

Innate Immunity

Macrophages and monocytes are highly heterologous cells that are distributed throughout the body. Macrophages process and present the antigens to elicit adaptive immune response. Due to their intrinsic phagocytic nature, macrophages can be easily targeted by surface engineered NPs, in which cognate ligands agonist to macrophage receptors can be conjugated on the NP surface (**Figure 1**). As discussed above several physico-chemical parameters of NPs such as size, surface charge, hydrophobicity, surface topography, and material composition can be optimized to facilitate the interactions between NPs and macrophage receptors (184–186). The rate of NP endocytosis also depends upon the type of cell surface receptors and the ligand conjugated to the NP surface. For example, NPs targeted via mannose and Fc receptors were rapidly internalized as compared to scavenger receptors (187). Endocytosis of IgG and anti-F4/80 antibody coated NPs showed more uptake rate and retention time inside the macrophages without affecting the cell viability (188, 189). Also, positively charged NPs interact more strongly with negatively charged phospholipid components of the cell membrane (190). Hyperactivation of some inflammatory cells can also be restricted through controlled release of stimulants using NPs. Upon activation, neutrophils can secrete variety of cytokines and hydrolytic enzymes in response to infection (191). Prolonged neutrophil activation often leads to acute inflammation and tissue damage at the localized site. Therefore, controlled release of molecules is

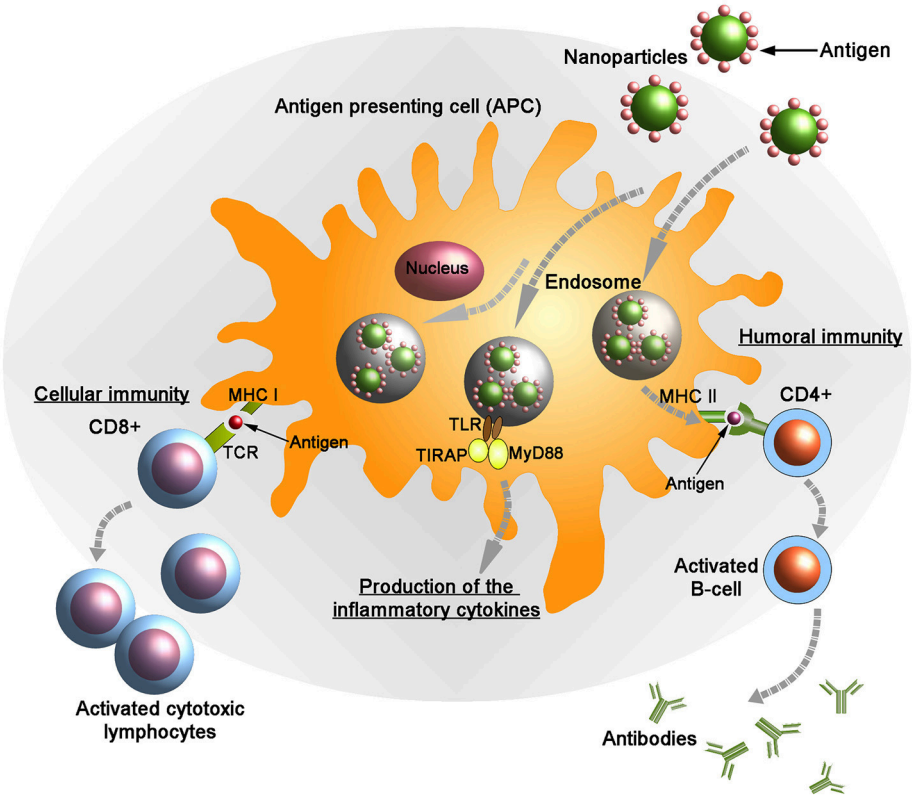

FIGURE 2 | Targeted delivery of antigenic molecules using surface engineered nanoparticles into the antigen presenting cells (APCs). Endogenously generated antigens are presented in complex with class I major histocompatibility complex (MHC I) on the membrane of APCs to CD8+ T lymphocytes. Following the interaction between MHC I and T-cell receptor (TCR) in presence of co-stimulatory molecules and cytokines the activated CD8+ cells kill the infected cells by inducing cytotoxicity. Also the antigens are presented on the APC surface by class II MHC molecules to the helper (CD4+) T cells. Subsequently, CD4+ cells activate B-cells that produce anti-microbial antibodies. Upon stimulation the adaptor proteins MyD88 (myeloid differentiation marker 88) and TIRAP (TIR domain containing adaptor protein) colocalize with TLR (toll-like receptor) allowing for activation of the NF-κB pathway and leading to the production of pro-inflammatory cytokines.

necessary to prevent the hyperactivation and massive recruitment of neutrophils. It has been reported that bovine serum albumin (BSA) NPs were able to modulate the functions of neutrophils following their internalization. Intravenous injection of anti-inflammatory peptide encapsulated polymeric NPs reduced neutrophil recruitment and subsequently hyperinflammation to prevent further tissue damage (192). The use of NPs to deliver vaccine/drugs in a controlled fashion is now considered as an attractive approach to develop therapeutic strategies against a range of acute and chronic inflammatory diseases (193).

Adpative Immunity

T and B-cells of the adaptive immune system express a repertoire of receptors to recognize a range of antigens. Activation or suppression of T-cell immunity can determine the fate of a disease. A number of NP based therapeutic strategies have been developed to regulate T-cell activity against viral, bacterial, or fungal infections. For example, antiviral siRNA or retroviral drug encapsulated lipid NPs or dendrimers were effectively delivered to $CD4^+$ T-cells to block HIV replication. This caused a significant reduction in HIV titer when compared with the use of non-encapsulated retroviral drugs (191, 194). T-cell activation also depends up on the type and size of NP used for the delivery of antigen. Liposome encapsulated antigens were better presented to $CD4^+$ T cells by APCs (195, 196) and delivery of 200 nm ova conjugated NPs increased MHC class I and II expression and also produced a higher percentage of antigen specific $CD4^+$T cells as compared to 30 nm ova conjugated particles (197).

B cells are able to recognize and respond to the microbial surface antigens through B-cell receptors (198). Activation and clonal expansion of antigen specific B-cells using engineered NPs have been exploited for the development of vaccines against different diseases (**Figure 2**). Encapsulation of antigen in virus like particles (VLPs) was able to induce strong and durable humoral responses when compared with the administration of exposed vaccine molecules (199). The potency of immune responses also depends upon the mode of antigen presentation to the target cells. Surface conjugated immunogenic proteins and peptides were able to activate B cells much stronger than encapsulated antigens (200). A single dose of PLGA NPs with surface displayed ovalbumin (OVA) elicited strong antibody responses *in vivo* as compared to free OVA (201, 202). NPs can also be used to activate specific immune responses. A study has shown that peptide conjugated carbon nanotubes

showed significant antigen specific IgG response in comparison to peptide or adjuvant alone (83).

NANOPARTICLES CAN BE USED TO INCREASE CROSS ANTIGEN PRESENTATION

In general, antigens captured by APCs from the extracellular environment are targeted to the endo-lysosomal compartments, where they are first processed into peptides and then loaded onto class II MHC molecules before presentation to $CD4^+$ helper T cells. However, cytosolic antigens are loaded on MHC class I molecules and presented to $CD8^+$ T-cells, which are crucial for the clearance of viral and intracellular infections (203). It is reported that some fraction of antigens delivered through NPs are trafficked to cytosolic vacuoles of APCs and presented by MHC class I molecules (203–205). The NP mediated cross antigen presentation was first demonstrated in antigens conjugated to iron oxide polymer NPs (206–209). In addition, inorganic and polymeric NPs have also been used for antigen delivery to cytosol (210–212). In this context, lipid NPs were shown to induce $CD8^+$ T cell expansion by efficient antigen cross presentation against viral infection in *in-vivo* models (102, 213). Similarly, invariant natural killer T cells (iNKT), which are a special subset of T-cells, recognize lipid antigens presented by CD1d cells. PLGA NPs conjugated with α-galactosylceramide glycolipid, an iNKT cell stimulant, increased cytokine release as well as expansion of antigen specific CD8+ T cells (214). The cross antigen presentation also depends upon the particle-antigen linkages. It has been shown that disulfide bonding between NP and antigens caused release of antigens into the endosomal compartment and also enhanced $CD8^+$ T cell formation as compared to non-degradable linkers (215, 216). Similarly, pulmonary administration of NPs efficiently enhanced cross antigen presentation, which resulted in at least 10-fold more effector $CD8^+$T cells in lungs (217).

NANOPARTICLES AS ADJUVANTS TO GENERATE IMMUNE RESPONSES IN LYMPHOID ORGANS

Adjuvants are known to enhance and prolong the immune responses against antigens. Delivery of adjuvants and antigens using NPs have been found useful to prolong their exposure in the lymphoid organs such as lymph nodes to generate robust immune responses. This is especially important for small adjuvant molecules, which are rapidly cleared from the bloodstream. NPs with a size ranging from 10–100 nm can penetrate the extracellular matrix and travel to the lymph nodes where they can be internalized by the resident macrophages to activate T-cell responses (218–220). The bio-distribution of NPs also depends upon the route of administration and size. It was observed that larger particles accumulated near the site of NPs and were subsequently endocytosed by the local APCs (160), whereas the smaller NPs drained to the blood capillaries (158, 218). PEG coated liposomes of 80–90 nm diameter showed higher accumulation in lymph nodes after subcutaneous administration as compared to intravenous and intraperitoneal administration (221).

CONCLUSIONS

The nano-immuno formulations can improve the antigen stability, targeted delivery and also enhance their immunogenicity properties. Most soluble antigens cannot be efficiently endocytosed by the APCs and hence are poorly effective in inducing protective immunity. The immunogenicity of such soluble vaccine antigens can be improved by conjugating them with nanocarriers that can facilitate the recognition and uptake by APCs. This strategy has already been proved effective for inducing/increasing the immunogenicity of poorly immunogenic antigens, such as polysaccharides of pneumococcal vaccines (222). In the last few years, the application of nanotechnology in the field of immune engineering is growing rapidly with a number of new carrier synthesis strategies. Furthermore, novel nano formulations also contain immunostimulatory molecules to enhance the adjuvant properties of the nanoparticles. Co-encapsulation of the TLR agonists [e.g., CpG, poly(I:C)] (77) or imiquimoid (78) into dextran or chitosan NPs, respectively enhanced receptor-based recognition of the nanovaccines with subsequent cell activation. The recent study by Margaroni et al. showed that vaccination with poly(D,L-lactide-co-glycolide; PLGA) nanoparticles with *Leishmania infantum* antigens (sLiAg) and surface-modified with a TNFα-mimicking eight-amino-acid peptide (p8) induced significant protection against parasite infection in BALB/c mice accompanied by activation of CD8+ T cells and increase in IFNγ production (223).

Additionally, NPs can be tailored for non-invasive administration and prolonged delivery of the vaccine antigens to a specific location, thus providing the possibility for formulation of the single dose vaccine. Several studies clearly demonstrated the efficacy of the non-invasively administered vaccines such as intranasal application of influenza nano vaccine (224), chitosan NPs with hemagglutinin protein of H1N1 influenza virus (225), *Streptococcus equi* proteins (226), hepatitis B surface antigen (pRc/CMV-HBs) (227) and plasmid encoding a multi-epitope protein against *M. tuberculosis* (pHSP65pep) (228) or antigen 85B (229) were used to provide protective immunity against infections. These considerations can improve the progress of ongoing strategies in the development of nanoparticle-based vaccines. In future, development of nanovaccines will address not only the possibility to induce the immune response but also the anti-infective therapeutic activity of NPs thus representing the feasibility to apply multifunctional particles for the treatment of diseases.

AUTHOR CONTRIBUTIONS

AS and RP wrote the manuscript. AS supervised the process. MS wrote the part on Use of nanocarriers in vaccine delivery to dendritic cells.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

EXHIBIT 382

White Paper on Studying the Safety of the Childhood Immunization Schedule

For the Vaccine Safety Datalink

National Center for Emerging and Zoonotic Infectious Diseases
Immunization Safety Office

Exhibit 382

White Paper on the Safety of the Childhood Immunization Schedule
Vaccine Safety Datalink
Centers for Disease Control and Prevention | 1600 Clifton Road | Atlanta GA 30329

Notice: The project that is the subject of this report was approved and funded by the Immunization Safety Office, Centers for Disease Control and Prevention. The contributors responsible for the content of the White Paper were funded by Task Order contract 200-2012-53582/0004 awarded as a prime contract to Kaiser Foundation Hospitals.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Additional copies of this report are available from Jason Glanz, PhD, Jason.M.Glanz@kp.org

1.5 Studying the Safety of the Childhood Immunization Schedule: Defining Key Concepts

The Immunization Schedule

The IOM committee acknowledged that, in order to study the safety of the childhood immunization schedule, more clarity was needed about what defines the schedule. The U.S. immunization schedule, established by the ACIP, is an extensive set of immunization recommendations guiding immunization delivery from birth through old age.[22,23] The immunization schedule changes over time, as new vaccines are licensed, or the recommendations for existing vaccines change based on new knowledge. In addition, for some vaccines, the immunization schedule allows for a relatively wide age interval within which vaccines can be delivered (e.g. the third dose of inactivated poliovirus vaccine [IPV] is recommended to be administered between 6 and 18 months of age).[23] Finally, the schedule also allows for the use of different vaccine products with different dosing schedules (e.g. there are two different rotavirus vaccines currently licensed in the U.S., one which requires two doses and another which requires three doses).

For the purposes of the White Paper, we chose to focus on the schedule of vaccines routinely recommended for infants and young children before 24 months of age. The rationale for this decision is as follows. First, more vaccines are recommended before 24 months of age than at any other time of life, with multiple doses of particular vaccines recommended. Second, parents appear to be more concerned about the safety of the schedule (i.e. the timing and spacing of multiple vaccines) for young children rather than for older children and adults.[11-14] Third, several of the medical conditions of concern to parents, such as asthma and allergic disorders, may become apparent clinically in the pre-school age group, roughly corresponding to 2 to 6 years of age. Finally, long periods of time elapse between the infant immunization series, the "school entry" series at 4 to 6 years of age, and the "pre-teenager" series at 11 years of age; these long time periods create conceptual as well as methodological uncertainty about what would define the schedule in later childhood and how it could be evaluated.

Safety

For the White Paper, we chose to explicitly define safety as the absence of vaccine-associated adverse events following immunization. Parental vaccine delay or refusal leads to an increased risk of vaccine-preventable disease in children,[16-18] and safety could be defined more broadly to include the prevention of disease. However, considerations related to vaccine effectiveness, and the risks associated with vaccine refusal, were considered out of scope of this White Paper. Nonetheless, any new knowledge generated about adverse events related to the immunization schedule could be used in the future by national policy makers when weighing all available evidence about the benefits and risks of vaccination.

Focus on Long-term Outcomes

While there is not a uniform definition of what constitutes a short- versus long-term adverse event, short-term adverse events are typically thought to occur in the hours, days, or weeks following vaccination. For example, VSD studies of vaccine safety will generally evaluate adverse events in the 1-2, 1-7, 1-14, or 1-42 days following vaccination. Long-term outcomes can be thought of as occurring in the months to years following vaccination.

After stakeholder engagement and a review of existing literature, the IOM committee concluded that while both short- and long-term adverse events were important, the study of long-term outcomes following the routine schedule was a higher priority. The current safety surveillance systems such as the VSD,[2] and the Post-Licensure Rapid Immunization Safety Monitoring (PRISM)[24] system of the Food and Drug Administration (FDA), already have extensive systems in place to assess short-term outcomes. Parents have expressed more concerns about long-term than short-term health outcomes, and have argued that long-term health outcomes have been less well-studied in the context of vaccine safety. Finally, because the childhood immunization schedule is essentially a long-term exposure, occurring over 18 to 24 months, long-term adverse events may be more biologically plausible than short-term events. Therefore, for the purposes of the White Paper we chose to focus primarily on long-term adverse events.

EXHIBIT 383

Vaccine Product Approval Process

FDA's Center for Biologics Evaluation and Research (CBER) is responsible for regulating vaccines in the United States. Current authority for the regulation of vaccines resides primarily in Section 351 of the Public Health Service Act and specific sections of the Federal Food, Drug and Cosmetic Act.

Vaccine clinical development follows the same general pathway as for drugs and other biologics. A sponsor who wishes to begin clinical trials with a vaccine must submit an Investigational New Drug application (IND) to FDA. The IND describes the vaccine, its method of manufacture, and quality control tests for release. Also included are information about the vaccine's safety and ability to elicit a protective immune response (immunogenicity) in animal testing, as well as the proposed clinical protocol for studies in humans.

Pre-marketing (pre-licensure) vaccine clinical trials are typically done in three phases, as is the case for any drug or biologic. Initial human studies, referred to as Phase 1, are safety and immunogenicity studies performed in a small number of closely monitored subjects. Phase 2 studies are dose-ranging studies and may enroll hundreds of subjects. Finally, Phase 3 trials typically enroll thousands of individuals and provide the critical documentation of effectiveness and important additional safety data required for licensing. At any stage of the clinical or animal studies, if data raise significant concerns about either safety or effectiveness, FDA may request additional information or studies, or may halt ongoing clinical studies.

If successful, the completion of all three phases of clinical development can be followed by the submission of a Biologics License Application (BLA). To be considered, the license application must provide the multidisciplinary FDA reviewer team (medical officers, microbiologists, chemists, biostatisticians, etc.) with the efficacy and safety information necessary to make a risk/benefit assessment and to recommend or oppose the approval of a vaccine. Also during this stage, the proposed manufacturing facility undergoes a pre-approval inspection during which production of the vaccine as it is in progress is examined in detail.

Following FDA's review of a license application for a new indication, the sponsor and the FDA may present their findings to FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC). This non-FDA expert committee (scientists, physicians, biostatisticians, and a consumer representative) provides advice to the Agency regarding the safety and efficacy of the vaccine for the proposed indication.

Vaccine approval also requires the provision of adequate product labeling to allow health care providers to understand the vaccine's proper use, including its potential benefits and risks, to communicate with patients and parents, and to safely deliver the vaccine to the public.

The FDA continues to oversee the production of vaccines after the vaccine and the manufacturing processes are approved, in order to ensure continuing safety. After licensure, monitoring of the product and of production activities, including periodic facility inspections, must continue as long as the manufacturer holds a license for the product. If requested by the FDA, manufacturers are required to submit to the FDA the results of their own tests for potency, safety, and purity for each vaccine lot. They may

also required to submit samples of each vaccine lot to the FDA for testing. However, if the sponsor describes an alternative procedure which provides continued assurance of safety, purity and potency, CBER may determine that routine submission of lot release protocols (showing results of applicable tests) and samples is not necessary.

Until a vaccine is given to the general population, all potential adverse events cannot be anticipated. Thus, many vaccines undergo Phase 4 studies-formal studies on a vaccine once it is on the market. Also, the government relies on the Vaccine Adverse Event Reporting System (VAERS) to identify problems after marketing begins. The VAERS system and how it works is discussed further on this website.

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Top ()

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Related Links from the Centers for Disease Control and Prevention

- What Would Happen If We Stopped Vaccinations (http://www.cdc.gov/vaccines/vac-gen/whatifstop.htm)
- Ten Things You Need To Know About Immunizations

(http://www.cdc.gov/vaccines/vac-gen/10-shouldknow.htm)

- CDC National Immunization Program (http://www.cdc.gov/vaccines/)

EXHIBIT 384

World Health Organization

Credits +

Global Vaccine Safety Summit

2 – 3 December 2019 | Geneva, Switzerland

Purpose of the event

The Global Vaccine Safety Summit is a 2-day event, from 2 to 3 December 2019, organized by the World Health Organization and held at the WHO's headquarters in Geneva, Switzerland.

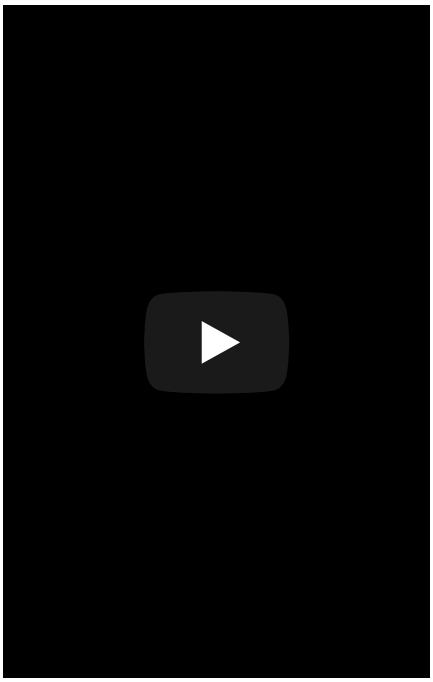
In the year that marks the 20th anniversary of the WHO's Global Advisory Committee on Vaccine Safety (GACVS), the Global Vaccine Safety Summit will be an opportunity to take stock of GACVS accomplishments and look towards priorities for the next decade.

At the Summit, WHO will present the Global Vaccine Safety Blueprint 2.0 strategy 2021-2030 to key stakeholders and collect their input for the final version, due for publication in the new year.

Attendees

Agenda

WHO works to ensure vaccinations are safe



Full screen

The Summit is meant for vaccine safety stakeholders from around the world, including current and former members of the Global Advisory Committee on Vaccine Safety (GACVS), immunisation programme managers, national regulatory authorities, pharmacovigilance staff from all WHO regions, and representatives of UN agencies, academic institutions, umbrella organizations of pharmaceutical companies, technical partners, industry representatives and funding agencies.

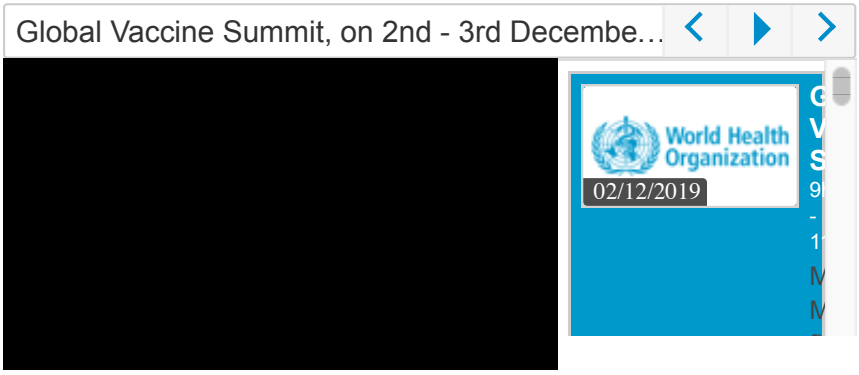


EXHIBIT 385

VITAMIN K_1
INJECTION

Phytonadione

Injectable Emulsion, USP

Aqueous Dispersion of Vitamin K_1

Ampul

R_x only

Protect from light. Keep ampuls

in tray until time of use.

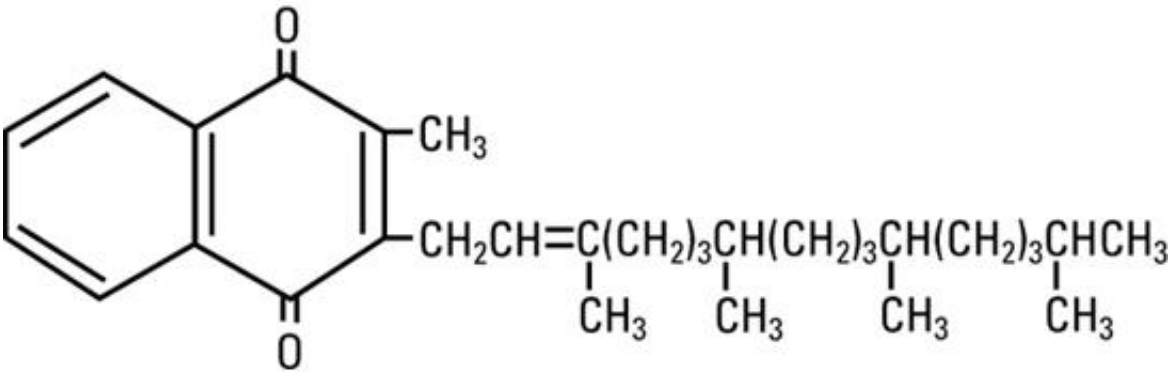
WARNING — INTRAVENOUS AND INTRAMUSCULAR USE

Severe reactions, including fatalities, have occurred during and immediately after INTRAVENOUS injection of phytonadione, even when precautions have been taken to dilute the phytonadione and to avoid rapid infusion. Severe reactions, including fatalities, have also been reported following INTRAMUSCULAR administration. Typically these severe reactions have resembled hypersensitivity or anaphylaxis, including shock and cardiac and/or respiratory arrest. Some patients have exhibited these severe reactions on receiving phytonadione for the first time. Therefore the INTRAVENOUS and INTRAMUSCULAR routes should be restricted to those situations where the subcutaneous route is not feasible and the serious risk involved is considered justified.

DESCRIPTION

Phytonadione is a vitamin, which is a clear, yellow to amber, viscous, odorless or nearly odorless liquid. It is insoluble in water, soluble in chloroform and slightly soluble in ethanol. It has a molecular weight of 450.70.

Phytonadione is 2-methyl-3-phytyl-1, 4-naphthoquinone. Its empirical formula is $C_{31}H_{46}O_2$ and its structural formula is:



Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) is a yellow, sterile, nonpyrogenic aqueous dispersion available for injection by the intravenous, intramuscular and subcutaneous routes. Each milliliter contains phytonadione 2 or 10 mg, polyoxyethylated fatty acid derivative 70 mg, dextrose, hydrous 37.5 mg in water for injection; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH is 6.3 (5.0 to 7.0). Phytonadione is oxygen sensitive.

CLINICAL PHARMACOLOGY

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) aqueous dispersion of vitamin K_1 for parenteral injection, possesses the same type and degree of activity as does naturally-occurring vitamin K, which is necessary for the production via the liver of active prothrombin (factor II), proconvertin (factor VII), plasma thromboplastin component (factor IX), and Stuart factor (factor X). The prothrombin test is sensitive to the levels of three of these four factors−II, VII, and X. Vitamin K is an essential cofactor for a microsomal enzyme that catalyzes the post-translational carboxylation of multiple, specific, peptide-bound glutamic acid residues in inactive hepatic precursors of factors II, VII, IX, and X. The resulting gamma-carboxy-glutamic acid residues convert the precursors into active coagulation factors that are subsequently secreted by liver cells into the blood.

Phytonadione is readily absorbed following intramuscular administration. After absorption, phytonadione is initially concentrated in the liver, but the concentration declines rapidly. Very little vitamin K accumulates in tissues. Little is known about the metabolic fate of vitamin K. Almost no free unmetabolized vitamin K appears in bile or urine.

In normal animals and humans, phytonadione is virtually devoid of pharmacodynamic activity. However, in animals and humans deficient in vitamin K, the pharmacological action of vitamin K is related to its normal physiological function, that is, to promote the hepatic biosynthesis of vitamin K dependent clotting factors.

The action of the aqueous dispersion, when administered intravenously, is generally detectable within an hour or two and hemorrhage is usually controlled within 3 to 6 hours. A normal prothrombin level may often be obtained in 12 to 14 hours.

In the prophylaxis and treatment of hemorrhagic disease of the newborn, phytonadione has demonstrated a greater margin of safety than that of the water-soluble vitamin K analogues.

INDICATIONS AND USAGE

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) is indicated in the following coagulation disorders which are due to faulty formation of factors II, VII, IX and X when caused by vitamin K deficiency or interference with vitamin K activity.

Vitamin K_1 Injection is indicated in:

- anticoagulant-induced prothrombin deficiency caused by coumarin or indanedione derivatives;
- prophylaxis and therapy of hemorrhagic disease of the newborn;
- hypoprothrombinemia due to antibacterial therapy;
- hypoprothrombinemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, celiac disease, intestinal resection, cystic fibrosis of the pancreas, and regional enteritis;
- other drug-induced hypoprothrombinemia where it is definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates.

CONTRAINDICATION

Hypersensitivity to any component of this medication.

WARNINGS

Benzyl alcohol as a preservative in Bacteriostatic Sodium Chloride Injection has been associated with toxicity in newborns. Data are unavailable on the toxicity of other preservatives in this age group. There is no evidence to suggest that the small amount of benzyl alcohol contained in Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP), when used as recommended, is associated with toxicity.

An immediate coagulant effect should not be expected after administration of phytonadione. It takes a minimum of 1 to 2 hours for measurable improvement in the prothrombin time. Whole blood or component therapy may also be necessary if bleeding is severe.

Phytonadione will not counteract the anticoagulant action of heparin.

When vitamin K_1 is used to correct excessive anticoagulant-induced hypoprothrombinemia, anticoagulant therapy still being indicated, the patient is again faced with the clotting hazards existing prior to starting the anticoagulant therapy. Phytonadione is not a clotting agent, but overzealous therapy with vitamin K_1 may restore conditions which originally permitted thromboembolic phenomena. Dosage should be kept as low as possible, and prothrombin time should be checked regularly as clinical conditions indicate.

Repeated large doses of vitamin K are not warranted in liver disease if the response to initial use of the vitamin is unsatisfactory. Failure to respond to vitamin K may indicate that the condition being treated is inherently unresponsive to vitamin K.

Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants.

WARNING: This product contains aluminum that may be toxic. Aluminum may reach toxic levels with prolonged parenteral administration if kidney function is impaired. Premature neonates are particularly at risk because their kidneys are immature, and they required large amounts of calcium and phosphate solutions, which contain aluminum.

Research indicates that patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 4 to 5 mcg/kg/day accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates of administration.

PRECAUTIONS

Drug Interactions

Temporary resistance to prothrombin-depressing anticoagulants may result, especially when larger doses of phytonadione are used. If relatively large doses have been employed, it may be necessary when reinstituting anticoagulant therapy to use somewhat larger doses of the prothrombin-depressing anticoagulant, or to use one which acts on a different principle, such as heparin sodium.

Laboratory Tests

Prothrombin time should be checked regularly as clinical conditions indicate.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies of carcinogenicity, mutagenesis or impairment of fertility have not been conducted with Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP).

Pregnancy

Animal reproduction studies have not been conducted with Vitamin K_1 Injection. It is also not known whether Vitamin K_1 Injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Vitamin K_1 Injection should be given to a pregnant woman only if clearly needed.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Vitamin K_1 Injection is administered to a nursing woman.

Pediatric Use

Hemolysis, jaundice, and hyperbilirubinemia in neonates, particularly those that are premature, may be related to the dose of Vitamin K_1 Injection. Therefore, the recommended dose should not be exceeded (See ADVERSE REACTIONS and DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

Deaths have occurred after intravenous and intramuscular administration. (See Box Warning.)

Transient "flushing sensations" and "peculiar" sensations of taste have been observed, as well as rare instances of dizziness, rapid and weak pulse, profuse sweating, brief hypotension, dyspnea, and cyanosis.

Pain, swelling, and tenderness at the injection site may occur.

The possibility of allergic sensitivity including an anaphylactoid reaction, should be kept in mind.

Infrequently, usually after repeated injection, erythematous, indurated, pruritic plaques have occurred; rarely, these have progressed to scleroderma-like lesions that have persisted for long periods. In other cases, these lesions have resembled erythema perstans.

Hyperbilirubinemia has been observed in the newborn following administration of phytonadione. This has occurred rarely and primarily with doses above those recommended (See PRECAUTIONS, *Pediatric Use*).

OVERDOSAGE

The intravenous LD_{50} of Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) in the mouse is 41.5 and 52 mL/kg for the 0.2% and 1% concentrations, respectively.

DOSAGE AND ADMINISTRATION

Whenever possible, Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) should be given by the subcutaneous route (See Box Warning). When intravenous administration is considered unavoidable, the drug should be injected very slowly, not exceeding 1 mg per minute.

Protect from light at all times.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Directions for Dilution

Vitamin K_1 Injection may be diluted with 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or 5% Dextrose and Sodium Chloride Injection. Benzyl alcohol as a preservative has been associated with toxicity in newborns. *Therefore, all of the above diluents should be preservative-free* (See WARNINGS). *Other diluents should not be used.* When dilutions are indicated, administration should be started immediately after mixture with the diluent, and unused portions of the dilution should be discarded, as well as unused contents of the ampul.

Prophylaxis of Hemorrhagic Disease of the Newborn

The American Academy of Pediatrics recommends that vitamin K_1 be given to the newborn. A single intramuscular dose of Vitamin K_1 Injection 0.5 to 1 mg within one hour of birth is recommended.

Treatment of Hemorrhagic Disease of the Newborn

Empiric administration of vitamin K_1 should not replace proper laboratory evaluation of the coagulation mechanism. A prompt response (shortening of the prothrombin time in 2 to 4 hours) following administration of vitamin K_1 is usually diagnostic of hemorrhagic disease of the newborn, and failure to respond indicates another

diagnosis or coagulation disorder.

Vitamin K_1 Injection 1 mg should be given either subcutaneously or intramuscularly. Higher doses may be necessary if the mother has been receiving oral anticoagulants.

Whole blood or component therapy may be indicated if bleeding is excessive. This therapy, however, does not correct the underlying disorder and Vitamin K_1 Injection should be given concurrently.

Anticoagulant-Induced Prothrombin Deficiency in Adults

To correct excessively prolonged prothrombin time caused by oral anticoagulant therapy—2.5 to 10 mg or up to 25 mg initially is recommended. In rare instances 50 mg may be required. Frequency and amount of subsequent doses should be determined by prothrombin time response or clinical condition (See WARNINGS). If in 6 to 8 hours after parenteral administration the prothrombin time has not been shortened satisfactorily, the dose should be repeated.

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) Summary of Dosage Guidelines (See circular text for details)

Newborns	**Dosage**
Hemorrhagic Disease of the Newborn Prophylaxis	0.5 to 1 mg IM within 1 hour of birth
Treatment	1 mg SC or IM (Higher doses may be necessary if the mother has been receiving oral anticoagulants)
Adults	**Initial Dosage**
Anticoagulant-Induced Prothrombin Deficiency (caused by coumarin or indanedione derivatives)	2.5 mg to 10 mg or up to 25 mg (rarely 50 mg)
Hypoprothrombinemia Due to other causes (Antibiotics; Salicylates or other drugs; Factors limiting absorption or synthesis)	2.5 mg to 25 mg or more (rarely up to 50 mg)

In the event of shock or excessive blood loss, the use of whole blood or component therapy is indicated.

Hypoprothrombinemia Due to Other Causes in Adults

A dosage of 2.5 to 25 mg or more (rarely up to 50 mg) is recommended, the amount and route of administration depending upon the severity of the condition and response obtained.

If possible, discontinuation or reduction of the dosage of drugs interfering with coagulation mechanisms (such as salicylates; antibiotics) is suggested as an alternative to administering concurrent Vitamin K_1 Injection. The severity of the coagulation disorder should determine whether the immediate administration of Vitamin K_1 Injection is required in addition to discontinuation or reduction of interfering drugs.

HOW SUPPLIED

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) is supplied in a package of 25 as follows:

Unit of Sale	Concentration

NDC 0409-9157-01 25 ampuls in a package	1 mg/0.5 mL (2 mg/mL)
NDC 0409-9158-01 25 ampuls in a package	10 mg/mL

Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

Protect from light. Keep ampuls in tray until time of use.

Distributed by Hospira, Inc., Lake Forest, IL 60045 USA

LAB-1141-1.0

Revised: 04/2018

PRINCIPAL DISPLAY PANEL - 0.5 mL Ampul Label - RL-7130

0.5 mL

NDC 0409-9157-31

Rx only

VITAMIN K_1 Inj.
Phytonadione Injectable
Emulsion, USP

1 mg/0.5 mL
Neonatal Concentration

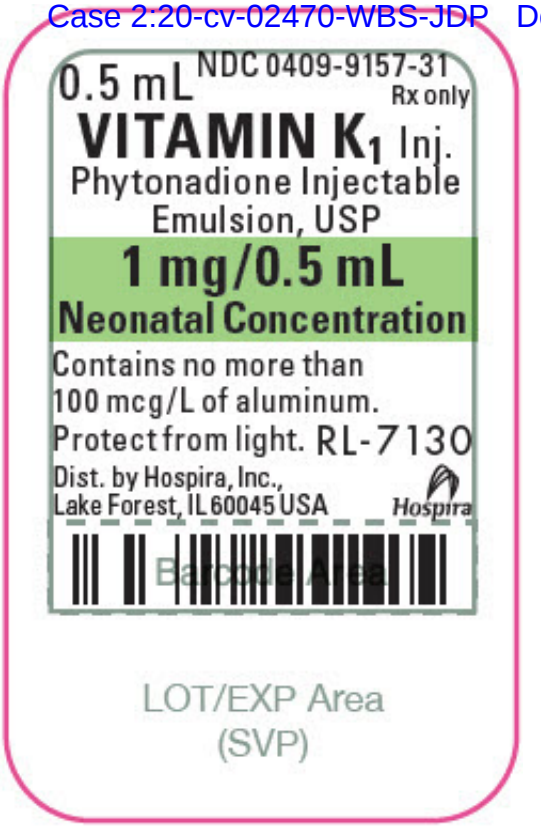
Contains no more than
100 mcg/L of aluminum.

Protect from light.

Dist. by Hospira, Inc.,
Lake Forest, IL 60045 USA

RL-7130

Hospira



PRINCIPAL DISPLAY PANEL - 0.5 mL Ampul Tray Label - RL-7129

0.5 mL Single-dose Ampul

Rx only NDC 0409-9157-50
Contains 5 of NDC 0409-9157-31

VITAMIN K_1 Injection
Phytonadione Injectable Emulsion, USP

1 mg/0.5 mL
Neonatal Concentration

Protect from light. Keep ampuls in tray until time of use. For Intramuscular, Subcutaneous, or Intravenous (with caution).

Each mL contains phytonadione 2 mg; polyoxyethylated fatty acid derivative 70 mg; dextrose, hydrous 37.5 mg; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH 6.3 (5.0 to 7.0). Usual dosage: See insert. Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

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Lake Forest, IL 60045 USA

Hospira

RL-7129

PRINCIPAL DISPLAY PANEL - 0.5 mL Ampul Label - RL-4148

NDC 0409-9157-25
Rx only

0.5 mL

VITAMIN K_1 Inj.
Phytonadione Injectable
Emulsion, USP

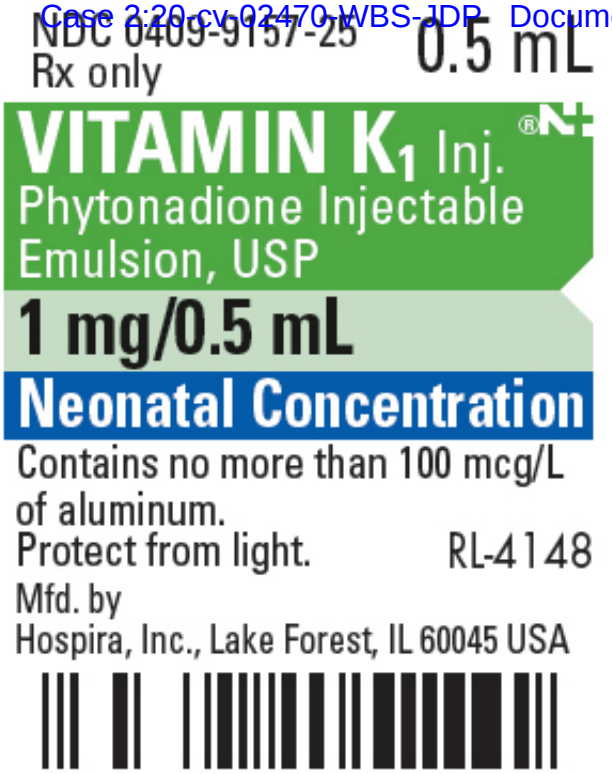
1 mg/0.5 mL

Neonatal Concentration

Contains no more than 100 mcg/L
of aluminum.

Protect from light.
RL-4148

Mfd. by
Hospira, Inc., Lake Forest, IL 60045 USA



PRINCIPAL DISPLAY PANEL - 0.5 mL Ampul Tray Label - RL-4149

5/**NDC** 0409-9157-25
Rx only
0.5 mL
Single-dose Ampul

VITAMIN K_1 Injection
Phytonadione Injectable Emulsion, USP

1 mg/0.5 mL

Neonatal Concentration

Protect from light. Keep ampuls in tray until time of use.
For I.M., S.C., or I.V. (with caution).

Each mL contains phytonadione 2 mg; polyoxyethylated fatty acid derivative 70 mg; dextrose, hydrous 37.5 mg; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH 6.3 (5.0 to 7.0). Usual dosage: See insert. Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

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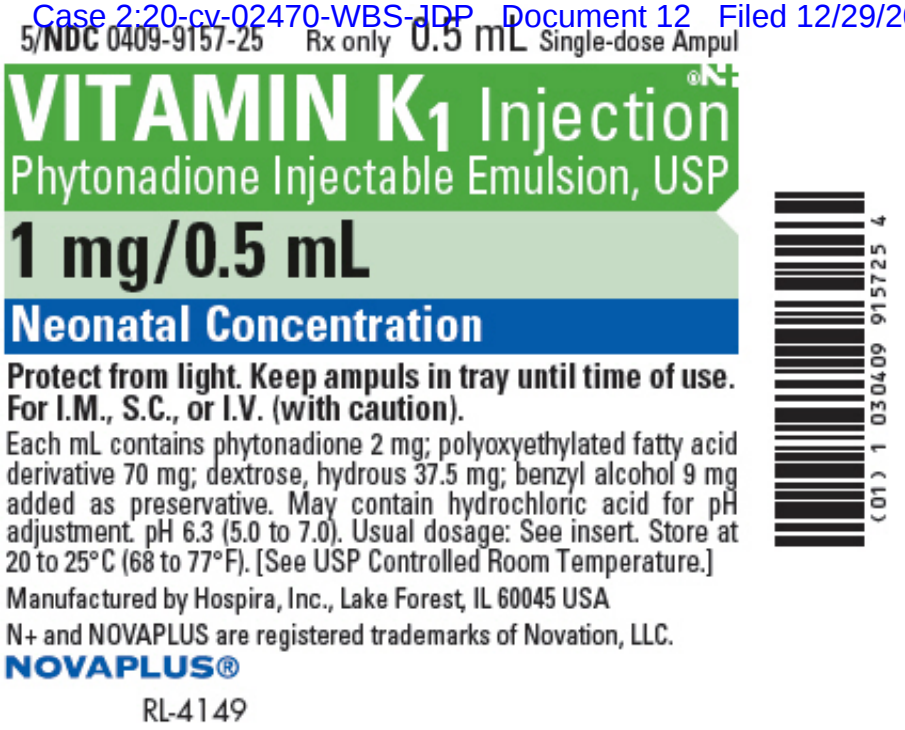
NOVAPLUS®

RL-4149



PRINCIPAL DISPLAY PANEL - 1 mL Ampul Label - RL-7126

1 mL only

NDC 0409-9158-31

VITAMIN K_1 Inj.

Phytonadione Injectable
Emulsion, USP

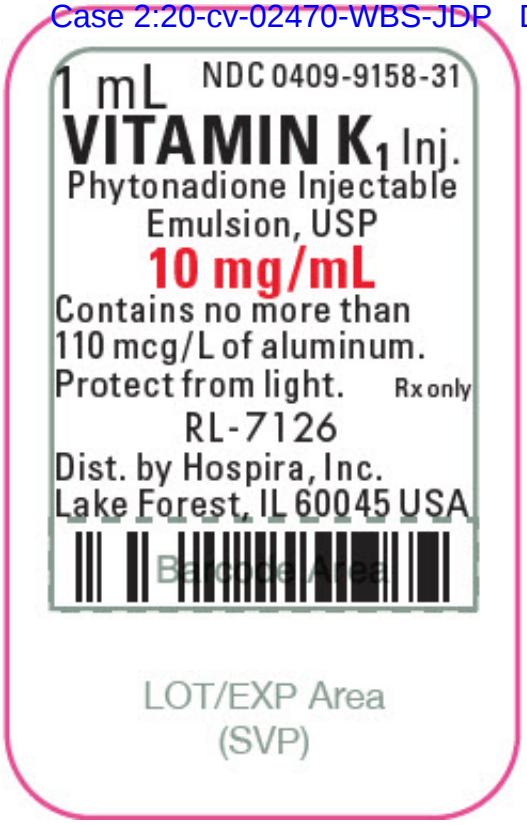
10 mg/mL

Contains no more than
110 mcg/L of aluminum.
Protect from light.

Rx only

RL-7126

Dist. by Hospira, Inc.
Lake Forest, IL 60045 USA



PRINCIPAL DISPLAY PANEL - 1 mL Ampul Tray Label - RL-7125

1 mL Single-dose Ampul

Rx only NDC 0409-9158-50
Contains 5 of NDC 0409-9158-31

VITAMIN K_1 Injection
Phytonadione Injectable Emulsion, USP

10 mg/mL

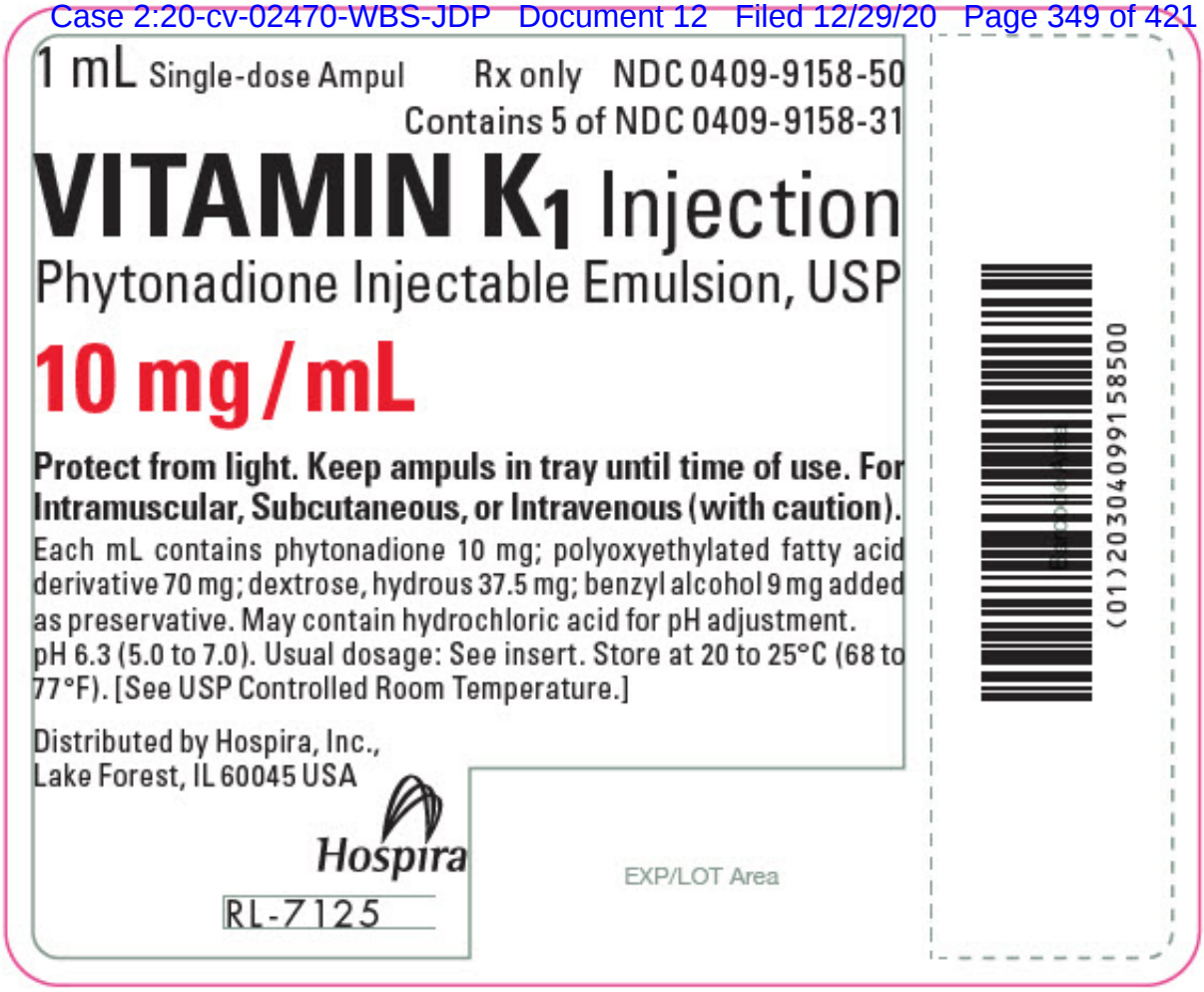
Protect from light. Keep ampuls in tray until time of use. For Intramuscular, Subcutaneous, or Intravenous (with caution).

Each mL contains phytonadione 10 mg; polyoxyethylated fatty acid derivative 70 mg; dextrose, hydrous 37.5 mg; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH 6.3 (5.0 to 7.0). Usual dosage: See insert. Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

Distributed by Hospira, Inc.,
Lake Forest, IL 60045 USA

Hospira

RL-7125



PRINCIPAL DISPLAY PANEL - 1 mL Ampul Label - RL-4150

NDC 0409-9158-25
Rx only

1 mL

VITAMIN K_1 Inj.
Phytonadione Injectable
Emulsion, USP

10 mg/mL

Contains no more than
110 mcg/L of aluminum.

Protect from light.
RL-4150

Mfd. by
Hospira, Inc., Lake Forest, IL 60045 USA

PRINCIPAL DISPLAY PANEL - 1 mL Ampul Tray Label - RL-4151

5/**NDC** 0409-9158-25
1 mL
Single-dose Ampul
Rx only

VITAMIN K_1 Injection
Phytonadione Injectable Emulsion, USP

10 mg/ mL

For I.M., S.C., or I.V. (with caution).

Protect from light. Keep ampuls in tray until time of use.

Each mL contains phytonadione 10 mg; polyoxyethylated fatty acid derivative 70 mg; dextrose, hydrous 37.5 mg; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH 6.3 (5.0 to 7.0). Usual dosage: See insert. Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

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NOVAPLUS®

RL-4151

5/NDC 0409-9158-25 1 mL Single-dose Ampul

Rx only

VITAMIN K_1 Injection

Phytonadione Injectable Emulsion, USP

10 mg/ mL

For I.M., S.C., or I.V. (with caution). ®N+

Protect from light. Keep ampuls in tray until time of use.

Each mL contains phytonadione 10 mg; polyoxyethylated fatty acid derivative 70 mg; dextrose, hydrous 37.5 mg; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH 6.3 (5.0 to 7.0). Usual dosage: See insert. Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

Manufactured by Hospira, Inc., Lake Forest, IL 60045 USA

NOVAPLUS®

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RL-4151

(01) 1 030409 915825 1

VITAMIN K1

phytonadione injection, emulsion

Product Information			
Product Type	HUMAN PRESCRIPTION DRUG	**Item Code (Source)**	NDC:0409-9157
Route of Administration	INTRAMUSCULAR, INTRAVENOUS, SUBCUTANEOUS		

Active Ingredient/Active Moiety		
Ingredient Name	**Basis of Strength**	**Strength**
PHYTONADIONE (UNII: A034SE7857) (PHYTONADIONE - UNII:A034SE7857)	PHYTONADIONE	2 mg in 1 mL

Inactive Ingredients	
Ingredient Name	**Strength**
POLYOXYL 35 CASTOR OIL (UNII: 6D4M1DAL6O)	70 mg in 1 mL
DEXTROSE MONOHYDRATE (UNII: LX22YL083G)	37.5 mg in 1 mL
WATER (UNII: 059QF0KO0R)	
BENZYL ALCOHOL (UNII: LKG8494WBH)	9 mg in 1 mL
HYDROCHLORIC ACID (UNII: QTT17582CB)	

Packaging				
#	**Item Code**	**Package Description**	**Marketing Start Date**	**Marketing End Date**
1	NDC:0409-9157-01	5 in 1 CONTAINER	06/22/2005	
1	NDC:0409-9157-50	5 in 1 TRAY		

1	NDC:0409-9157-31	0.5 mL in 1 AMPULE; Type 0: Not a Combination Product		
2	NDC:0409-9157-25	5 in 1 CONTAINER	06/26/2014	11/01/2019
2		5 in 1 TRAY		
2		0.5 mL in 1 AMPULE; Type 0: Not a Combination Product		

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA087954	06/22/2005	

VITAMIN K1

phytonadione injection, emulsion

Product Information

Product Type	HUMAN PRESCRIPTION DRUG	**Item Code (Source)**	NDC:0409-9158
Route of Administration	INTRAMUSCULAR, INTRAVENOUS, SUBCUTANEOUS		

Active Ingredient/Active Moiety

Ingredient Name	Basis of Strength	Strength
PHYTONADIONE (UNII: A034SE7857) (PHYTONADIONE - UNII:A034SE7857)	PHYTONADIONE	10 mg in 1 mL

Inactive Ingredients

Ingredient Name	Strength
POLYOXYL 35 CASTOR OIL (UNII: 6D4M1DAL6O)	70 mg in 1 mL
DEXTROSE MONOHYDRATE (UNII: LX22YL083G)	37.5 mg in 1 mL
WATER (UNII: 059QF0KO0R)	
BENZYL ALCOHOL (UNII: LKG8494WBH)	9 mg in 1 mL
HYDROCHLORIC ACID (UNII: QTT17582CB)	

Packaging

#	Item Code	Package Description	Marketing Start Date	Marketing End Date
1	NDC:0409-9158-01	5 in 1 CONTAINER	07/27/2005	
1	NDC:0409-9158-50	5 in 1 TRAY		
1	NDC:0409-9158-31	1 mL in 1 AMPULE; Type 0: Not a Combination Product		
2	NDC:0409-9158-25	5 in 1 CONTAINER	03/21/2014	02/01/2020
2		5 in 1 TRAY		
2		1 mL in 1 AMPULE; Type 0: Not a Combination Product		

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA087955	07/27/2005	

Labeler - Hospira, Inc. (141588017)

Establishment

Name	Address	ID/FEI	Business Operations
Hospira, Inc.		093132819	ANALYSIS(0409-9157, 0409-9158) , LABEL(0409-9157, 0409-9158) , MANUFACTURE(0409-9157, 0409-9158) , PACK(0409-9157, 0409-9158)

Establishment

Name	Address	ID/FEI	Business Operations
Hospira, Inc.		827731089	ANALYSIS(0409-9157, 0409-9158)

Establishment

Name	Address	ID/FEI	Business Operations
Hospira, Inc.		030606222	ANALYSIS(0409-9157, 0409-9158)

Revised: 12/2019 Hospira, Inc.

EXHIBIT 386

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use AquaMEPHYTON safely and effectively. See full prescribing information for AquaMEPHYTON.

AQUAMEPHYTON (phytonadione) injection, for intravenous, intramuscular, and subcutaneous use.
Initial U.S. Approval: 1960

WARNING – HYPERSENSITIVITY REACTIONS WITH INTRAVENOUS AND INTRAMUSCULAR USE
See full prescribing information for complete boxed warning.

Fatal hypersensitivity reactions, including anaphylaxis, have occurred during and immediately after INTRAVENOUS and INTRAMUSCULAR injection of AquaMEPHYTON. Reactions have occurred despite dilution to avoid rapid infusion and upon first and subsequent doses. Avoid the intravenous and intramuscular routes of administration unless the subcutaneous route is not feasible and the serious risk is justified (5.1)

----------RECENT MAJOR CHANGES----------

Warnings and Precautions, Cutaneous Reactions (5.3) 03/2018

INDICATIONS AND USAGE

AquaMEPHYTON is a vitamin K replacement indicated for the treatment of the following coagulation disorders which are due to faulty formation of factors II, VII, IX and X when caused by vitamin K deficiency or interference with vitamin K activity.

- Anticoagulant-induced hypoprothrombinemia deficiency caused by coumarin or indanedione derivatives; (1.1)
- Hypoprothrombinemia due to antibacterial therapy; (1.1)
- Hypoprothrombinemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, celiac disease, intestinal resection, cystic fibrosis of the pancreas, and regional enteritis; (1.1)
- Other drug-induced hypoprothrombinemia where is it definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates. (1.1)

AquaMEPHYTON is indicated for prophylaxis and treatment of vitamin K-deficiency bleeding in neonates. (1.2)

DOSAGE AND ADMINISTRATION

- Administer AquaMEPHYTON by the subcutaneous route, whenever possible. (2.1)
- When intravenous administration is unavoidable, inject the drug very slowly, not exceeding 1 mg per minute. (2.1)

DOSAGE FORMS AND STRENGTHS

Injection: 2 mg/mL and 10 mg/mL single -dose ampuls. (3)

CONTRAINDICATIONS

Hypersensitivity to any component of this medication. (4)

WARNINGS AND PRECAUTIONS

- Risk of Serious Adverse Reactions in Infants due to Benzyl Alcohol Preservative: Use benzyl alcohol-free formulations in neonates and infants, if available. (5.1)
- Cutaneous Reactions: May occur with parenteral use. Discontinue drug and manage medically. (5.3)

ADVERSE REACTIONS

Most common adverse reactions are cyanosis, diaphoresis, dizziness, dysgeusia, dyspnea, flushing, hypotension and tachycardia. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Teligent Pharma, Inc. at 1-856-697-1441, or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Anticoagulants: May induce temporary resistance to prothrombin-depressing anticoagulants. (7)

USE IN SPECIFIC POPULATIONS

- Pregnancy: If available, use the preservative-free formulation in pregnant women. (8.1)
- Lactation: If available, use the preservative-free formulation in lactating women. (8.2)
- Pediatric Use: The safety and effectiveness of AquaMEPHYTON in pediatric patients from 6 months to 17 years have not been established. (8.4)

Revised: 03/2018

FULL PRESCRIBING INFORMATION: CONTENTS *

BOXED WARNING
1 INDICATIONS & USAGE
1.1 Treatment of Hypoprothrombinemia Due to Vitamin K Deficiency or Interference
1.2 Prophylaxis and Treatment of Vitamin K-Deficiency Bleeding in Neonates
2 DOSAGE AND ADMINISTRATION
2.1 Dosing Considerations
2.2 Recommended Dosage for Coagulation Disorders from Vitamin K Deficiency of Interference
2.3 Recommended Dosage for Prophylaxis and Treatment of Vitamin K Deficiency Bleeding in Neonates
2.4 Directions for Dilution
3 DOSAGE FORMS AND STRENGTHS
4 CONTRAINDICATIONS
5 WARNINGS AND PRECAUTIONS
5.1 Hypersensitivity Reactions
5.2 Risk of Serious Adverse Reaction in Infants due to Benzyl Alcohol Preservative
5.3 Cutaneous Reactions
6 ADVERSE REACTIONS
6.1 Clinical Trials and Post Marketing experience
7 DRUG INTERACTIONS
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10 OVERDOSAGE
11 DESCRIPTION
12 CLINICAL PHARMACOLOGY
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12.2 Pharmacodynamics
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13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
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17 PATIENT COUNSELING INFORMATION

*** Sections or subsections omitted from the full prescribing information are not listed**

FULL PRESCRIBING INFORMATION

WARNING — HYPERSENSITIVITY REACTIONS WITH INTRAVENOUS AND INTRAMUSCULAR USE

Fatal hypersensitivity reactions, including anaphylaxis, have occurred during and immediately after intravenous and intramuscular injection of AquaMEPHYTON. Reactions have occurred despite dilution to avoid rapid intravenous infusion and upon first dose. Avoid the intravenous and intramuscular routes of administration unless the subcutaneous route is not feasible and the serious risk is justified *[see Warnings and Precautions (5.1)]*.

1 INDICATIONS AND USAGE

1.1 Treatment of Hypoprothrombinemia Due to Vitamin K Deficiency or Interference

AquaMEPHYTON is indicated for the treatment of the following coagulation disorders which are due to faulty formation of factors II, VII, IX and X when caused by vitamin K deficiency or interference with vitamin K activity:

- anticoagulant-induced hypoprothrombinemia caused by coumarin or indanedione derivatives;
- hypoprothrombinemia due to antibacterial therapy;
- hypoprothrombinemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, celiac disease, intestinal resection, cystic fibrosis of the pancreas, and regional enteritis;
- other drug-induced hypoprothrombinemia where it is definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates.

1.2 Prophylaxis and Treatment of Vitamin K-Deficiency Bleeding in Neonates

AquaMEPHYTON is indicated for prophylaxis and treatment of vitamin K-deficiency bleeding in neonates

2 DOSAGE AND ADMINISTRATION

2.1 Dosing Considerations

Whenever possible, administer AquaMEPHYTON by the subcutaneous route *[see Boxed Warning]*. When intravenous administration is unavoidable, inject the drug very slowly, not exceeding 1 mg per minute *[see Warnings and Precautions (5.1)]*.

Monitor international normalized ratio (INR) regularly and as clinical conditions indicate. Use the lowest effective dose of AquaMEPHYTON.

The coagulant effects of AquaMEPHYTON are not immediate; improvement of INR may take 1-8 hours. Interim use of whole blood or component therapy may also be necessary if bleeding is severe.

Whenever possible, administer benzyl alcohol-free formulations in pediatric patients *[see Warnings and Precautions (5.2), Use in Specific Populations (8.4)]*.

When AquaMEPHYTON is used to correct excessive anticoagulant-induced hypoprothrombinemia, anticoagulant therapy still being indicated, the patient is again faced with the clotting hazards existing prior to starting the anticoagulant therapy. AquaMEPHYTON is not a clotting agent, but overzealous therapy with AquaMEPHYTON may restore conditions which originally permitted thromboembolic phenomena. Dosage should be kept as low as possible, and INR should be checked regularly as clinical conditions indicate.

2.2 Recommended Dosage for Coagulation Disorders from Vitamin K Deficiency or Interference

The recommended dosage of AquaMEPHYTON is based on whether the hypoprothrombinemia is anticoagulant-induced (e.g., due to coumarin or indanedione derivatives) or non-anticoagulant-induced (e.g., due to antibiotics; salicylates or other drugs; factors limiting absorption or synthesis) as follows:

- Anticoagulant-Induced Hypoprothrombinemia: AquaMEPHYTON 2.5 mg to 10 mg or more subcutaneously, intramuscularly, or intravenously. Up to 25 mg to 50 mg may be administered as a single dose.

  Repeated large doses of AquaMEPHYTON are not warranted in liver disease if the initial response is unsatisfactory. Failure to respond to AquaMEPHYTON may indicate that the condition being treated is inherently unresponsive to AquaMEPHYTON.

- Hypoprothrombinemia Due to Other Causes (Non-Anticoagulation-Induced Hypoprothrombinemia): AquaMEPHYTON 2.5 mg to 25 mg or more intravenously, intramuscularly, or subcutaneously. Up to 50 mg may be administered as a single dose.

Evaluate INR after 6-8 hours, and repeat dose if INR remains prolonged. Modify subsequent dosage (amount and frequency) based on the INR or clinical condition.

2.3 Recommended Dosage for Prophylaxis and Treatment of Vitamin K Deficiency Bleeding in Neonates

Prophylaxis of Vitamin K-Deficiency Bleeding in Neonates
The recommended dosage of AquaMEPHYTON is 0.5 mg to 1 mg within one hour of birth for a single dose.

Treatment of Vitamin K Deficiency Bleeding in Neonates
The recommended dosage of AquaMEPHYTON is 1 mg given either subcutaneously or intramuscularly. Consider higher doses if the mother has been receiving oral anticoagulants.

A failure to respond (shortening of the INR in 2 to 4 hours) may indicate another diagnosis or coagulation disorder.

2.4 Directions for Dilution
Dilute AquaMEPHYTON with 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or 5% Dextrose and Sodium Chloride Injection. Avoid use of other diluents that may contain benzyl alcohol, which can cause serious toxicity in newborns or low birth weight infants *[see Warnings and Precautions (5.2)* and *Use in Specific Populations (8.4)*.
When diluted, start administration of AquaMEPHYTON immediately after dilution.
Discard unused portions of diluted solution as well as unused contents of the ampul.

Protect AquaMEPHYTON from light at all times.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

3 DOSAGE FORMS AND STRENGTHS
Injection: 2 mg/mL and 10 mg/mL single-dose ampuls.

4 CONTRAINDICATIONS
Hypersensitivity to phytonadione or any other component of this medication *[see Warnings and Precautions (5.1)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity Reactions

Fatal and severe hypersensitivity reactions, including anaphylaxis, have occurred with intravenous or intramuscular administration of AquaMEPHYTON. Reactions have occurred despite dilution to avoid rapid intravenous infusion and upon first dose. These reactions have included shock, cardiorespiratory arrest, flushing, diaphoresis, chest pain, tachycardia, cyanosis, weakness, and dyspnea. Administer AquaMEPHYTON subcutaneously whenever feasible. Avoid the intravenous and intramuscular routes of administration unless the subcutaneous route is not feasible and the serious risk is justified *[see Dosage and Administration (2.1)]*.

5.2 Risk of Serious Adverse Reaction in Infants due to Benzyl Alcohol Preservative

Use benzyl alcohol-free formulations in neonates and infants, if available. Serious and fatal adverse reactions including "gasping syndrome" can occur in neonates and infants treated with benzyl alcohol-preserved drugs, including AquaMEPHYTON. The "gasping syndrome" is characterized by central nervous system depression, metabolic acidosis, and gasping respirations.

When prescribing AquaMEPHYTON in infants, consider the combined daily metabolic load of benzyl alcohol from all sources including AquaMEPHYTON (contains 9 mg of benzyl alcohol per mL) and other drugs containing benzyl alcohol. The minimum amount of benzyl alcohol at which serious adverse reactions may occur is not known *[see Use in Specific Populations (8.1, 8.2 and 8.4)]*.

5.3 Cutaneous Reactions

Parenteral administration of vitamin K replacements (including AquaMEPHYTON) may cause cutaneous reactions. Reactions have included eczematous reactions, scleroderma-like patches, urticaria, and delayed-type hypersensitivity reactions. Time of onset ranged from 1 day to a year after parenteral administration. Discontinue AquaMEPHYTON for skin reactions and institute medical management.

6 ADVERSE REACTIONS

The following serious adverse reactions are described elsewhere in the labeling:

- Hypersensitivity Reactions *[see Warnings and Precautions (5.1)]*
- Cutaneous Reactions *[see Warnings and Precautions (5.3)]*

6.3 Clinical Trials and Post-Marketing Experience

The following adverse reactions associated with the use of AquaMEPHYTON were identified in clinical studies or postmarketing reports. Because some of these reactions were reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Cardiac Disorders: Tachycardia, hypotension.
General disorders and administration site conditions: Generalized flushing; pain, swelling, and tenderness at injection site.
Hepatobiliary Disorders: Hyperbilirubinemia
Immune System Disorders: Fatal hypersensitivity reactions, anaphylactic reactions.
Neurologic: Dysgeusia, dizziness.
Pulmonary: Dyspnea.
Skin and Subcutaneous Tissue Disorders: Erythema, pruritic plaques, scleroderma-like lesions, erythema perstans.
Vascular: Cyanosis.

7 DRUG INTERACTIONS

Anticoagulants

AquaMEPHYTON may induce temporary resistance to prothrombin-depressing anticoagulants, especially when larger doses of AquaMEPHYTON are used. Should this occur, higher doses of anticoagulant therapy may be needed when resuming anticoagulant therapy, or a change in therapy to a different class of anticoagulant may be necessary (i.e., heparin sodium).

AquaMEPHYTON does not affect the anticoagulant action of heparin.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

AquaMEPHYTON contains benzyl alcohol, which has been associated with gasping syndrome in neonates. The preservative benzyl alcohol can cause serious adverse events and death when administered intravenously to neonates and infants. If AquaMEPHYTON is needed during pregnancy, consider using a benzyl alcohol-free formulation *[see Warnings and Precautions (5.2), Use in Specific Populations (8.4)]*.

Published studies with the use of phytonadione during pregnancy have not reported a clear association with phytonadione and adverse developmental outcomes *(see Data)*. There are maternal and fetal risks associated with vitamin K deficiency during pregnancy *(see Clinical Considerations)*. Animal reproduction studies have not been conducted with phytonadione.

The estimated background risk for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

Pregnant women with vitamin K deficiency hypoprothrombinemia may be at an increased risk for bleeding diatheses during pregnancy and hemorrhagic events at delivery. Subclinical maternal vitamin K deficiency during pregnancy has been implicated in rare cases of fetal intracranial hemorrhage.

Data

Human Data

Phytonadione has been measured in cord blood of infants whose mothers were treated with phytonadione during pregnancy in concentrations lower than seen in maternal plasma. Administration of vitamin K_1 to pregnant women shortly before delivery increased both maternal and cord blood concentrations. Published data do not report a clear association with phytonadione and adverse maternal or fetal outcomes when used during pregnancy. However, these studies cannot definitively establish the absence of any risk because of methodologic limitations including small sample size and lack of blinding.

Animal Data

In pregnant rats receiving vitamin K_1 orally, fetal plasma and liver concentrations increased following administration, supporting placental transfer.

8.2 Lactation

Risk Summary

AquaMEPHYTON contains benzyl alcohol. If available, preservative-free AquaMEPHYTON is recommended when AquaMEPHYTON is needed during lactation *[see Warnings and Precautions (5.2), Use in Specific Populations (8.4)]*.

Phytonadione is present in breastmilk. There are no data on the effects of AquaMEPHYTON on the breastfed child or on milk production. The developmental and health benefits of breastfeeding should be considered along with the clinical need for AquaMEPHYTON and any potential adverse effects on the breastfed child from AquaMEPHYTON or from the underlying maternal condition.

8.4 Pediatric Use

The safety and effectiveness of AquaMEPHYTON for prophylaxis and treatment of vitamin K deficiency have been established in neonates. Use of phytonadione injection for prophylaxis and treatment of vitamin K deficiency is based on published clinical studies.

Serious adverse reactions including fatal reactions and the "gasping syndrome" occurred in premature neonates and infants in the intensive care unit who received drugs containing benzyl alcohol as a preservative. In these cases, benzyl alcohol dosages of 99 to 234 mg/kg/day produced high levels of benzyl alcohol and its metabolites in the blood and urine (blood levels of benzyl alcohol were 0.61 to 1.378 mmol/L). Additional adverse reactions included gradual neurological deterioration, seizures, intracranial hemorrhage, hematologic abnormalities, skin breakdown, hepatic and renal failure, hypotension, bradycardia, and cardiovascular collapse. Preterm, low-birth weight infants may be more likely to develop these reactions because they may be less able to metabolize benzyl alcohol.

When prescribing AquaMEPHYTON in infants consider the combined daily metabolic load of benzyl alcohol from all sources including AquaMEPHYTON (AquaMEPHYTON contains 9 mg of benzyl alcohol per mL) and other drugs containing benzyl alcohol. The minimum amount of benzyl alcohol at which serious adverse reactions may occur is not known *[see Warnings and Precautions (5.2)]*.

Whenever possible, use preservative-free phytonadione formulations in neonates. The preservative benzyl alcohol has been associated with serious adverse events and death in pediatric patients. Premature and low-birth weight infants may be more likely to develop toxicity.

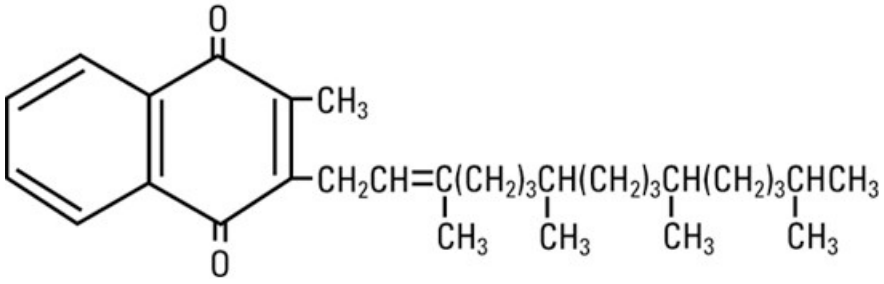
10 OVERDOSAGE

Hemolysis, jaundice, and hyperbilirubinemia in newborns, particularly in premature infants, may result from AquaMEPHYTON overdose.

11 DESCRIPTION

Phytonadione is a vitamin K replacement, which is a clear, yellow to amber, viscous, odorless or nearly odorless liquid. It is insoluble in water, soluble in chloroform and slightly soluble in ethanol. It has a molecular weight of 450.70.

Phytonadione is 2-methyl-3-phytyl-1, 4-naphthoquinone. Its empirical formula is $C_{31}H_{46}O_2$ and its molecular structure is:



AquaMEPHYTON injection is a yellow, sterile, aqueous colloidal solution of vitamin K_1, with a pH of 5.0 to 7.0, available for injection by the intravenous, intramuscular, and subcutaneous routes. AquaMEPHYTON is available in 1 mg (2 mg/mL) and 10 mg (10 mg/mL) single-dose ampuls. Each milliliter of AquaMEPHYTON contains the following inactive ingredients: 70 mg polyoxyethylated fatty acid derivative, 37.5 mg dextrose, 9 mg benzyl alcohol (preservative), and water for injection. AquaMEPHYTON may contain glacial acetic acid for pH adjustment to 6.3 (5.0 – 7.0).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

AquaMEPHYTON aqueous colloidal solution of vitamin K_1 for parenteral injection, possesses the same type and degree of activity as does naturally-occurring vitamin K, which is necessary for the production via the liver of active prothrombin (factor II), proconvertin (factor VII), plasma thromboplastin component (factor IX), and Stuart factor (factor X). Vitamin K is an essential cofactor for a microsomal enzyme that catalyzes the post-translational carboxylation of multiple, specific, peptide-bound glutamic acid residues in inactive hepatic precursors of factors II, VII, IX, and X. The resulting gamma-carboxy-glutamic acid residues convert the precursors into active coagulation factors that are subsequently secreted by liver cells into the blood.

In normal animals and humans, phytonadione is virtually devoid of activity. However, in animals and humans deficient in vitamin K, the pharmacological action of vitamin K is related to its normal physiological function, that is, to promote the hepatic biosynthesis of vitamin K dependent clotting factors.

12.2 Pharmacodynamics

The action of the aqueous dispersion, when administered intravenously, is generally detectable within an hour or two and hemorrhage is usually controlled within 3 to 6 hours. A normal INR may often be obtained in 12 to 14 hours.

12.3 Pharmacokinetics

Absorption:
Phytonadione is readily absorbed following intramuscular administration.

Distribution:
After absorption, phytonadione is initially concentrated in the liver, but the concentration declines rapidly. Very little vitamin K accumulates in tissues.

Elimination:
Little is known about the metabolic fate of vitamin K. Almost no free unmetabolized vitamin K appears in bile or urine.

13 NONCLINICAL TOXICOLOGY SECTION

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies of carcinogenicity, genotoxicity or impairment of fertility have not been conducted with phytonadione.

16 HOW SUPPLIED/STORAGE AND HANDLING

AquaMEPHYTON is a yellow, sterile, aqueous colloidal solution and is supplied in a package of 25 as follows:

NDC No.	Container	Amount of AquaMEPHYTON® In Container	Volume	Concentration
52565-092-05	1 mL single-dose ampul	1 mg	0.5 mL	2 mg/mL
52565-093-05	1 mL single-dose ampul	10 mg	1 mL	10 mg/mL

Store at 20° to 25°C (68° to 77°F); excursions permitted to 15° to 30°C (59° to 86°F) [see USP Controlled Room Temperature].

Protect AquaMEPHYTON from light. Store container in closed original carton until contents have been used.

17 PATIENT COUNSELING INFORMATION

Inform the patient of the following important risks of AquaMEPHYTON:

Serious Hypersensitivity Reactions
Advise the patient and caregivers to immediately report signs of hypersensitivity after receiving AquaMEPHYTON *[see Warnings and Precautions (5.1)]*.

Risk of Gasping Syndrome Due to Benzyl Alcohol
Advise the patient and caregivers of the risk of gasping syndrome associated with the use of products that contain benzyl alcohol (including AquaMEPHYTON) in neonates, infants, and pregnant women *[see Warnings and Precautions (5.2)]*.

Cutaneous Reactions
Advise the patient and caregivers to report the occurrence of new rashes after receiving AquaMEPHYTON. These reactions may be delayed for up to a year after treatment *[see Warnings and Precautions (5.3)]*.

Manufactured by:
Valdepharm
Val De Reuil 27100 France

Distributed by:
Teligent Pharma, Inc.
Buena, NJ 08310

EXHIBIT 387

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Immunization Schedules

For Health Care Providers

Child and Adolescent Immunization Schedule (birth through 18 years)

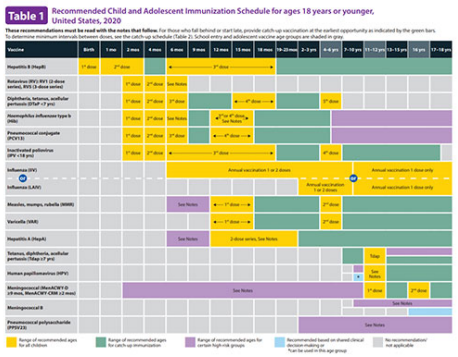


Table 1 Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2020

Adult Immunization Schedule (19 years and older)

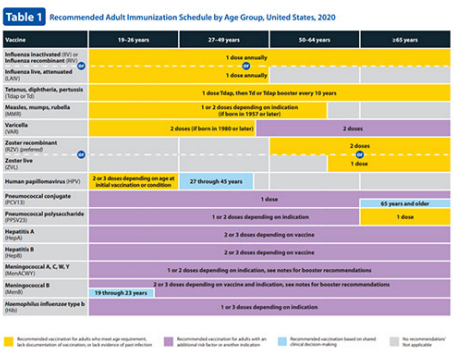
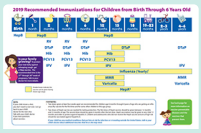


Table 1 Recommended Adult Immunization Schedule by Age Group, United States, 2020

Resources for Health Care Providers

For Parents & Adults



Parent-Friendly Schedule for Infants and Children (birth-6 years)

Parent-Friendly Schedule for Preteens and Teens (7-18 years)

Resources for Parents

Resources for Adults

Related Pages

- Vaccines and Immunizations
- Advisory Committee on Immunization Practices (ACIP)
- Vaccine Information Statements

Download “CDC Vaccine Schedules” free for iOS and Android devices.

Order Hard Copies

Hard copies of the schedule are available for free using the CDC-info on Demand order form.

Page last reviewed: February 3, 2020
Content source: National Center for Immunization and Respiratory Diseases

EXHIBIT 388

USP Therapeutic Categories Model Guidelines

Therapeutic Category	Pharmacologic Class	Formulary Key Drug Types
Analgesics	*Non-opioid Analgesics*	
	Opioid Analgesics	Opioid Analgesics, Long-acting
		Opioid Analgesics, Short-acting
Anesthetics	Local Anesthetics	-
Antibacterials	Aminoglycosides	
	Beta-lactam, Cephalosporins	Cephalosporin Antibacterials, 1st Generation
		Cephalosporin Antibacterials, 2nd Generation
		Cephalosporin Antibacterials, 3rd Generation
		Cephalosporin Antibacterials, 4th Generation
	Beta-lactam, Penicillins	Amino Derivative Penicillins
		Extended Spectrum Penicillins
		Natural Penicillins
		Penicillinase-resistant Penicillins
	Beta-lactam, Other	-
	Macrolides	Erythromycins
		Ketolides
		Macrolides (Non-erythromycins, Non-ketolides)
	Quinolones	-

	Sulfonamides	
	Tetracyclines	
	Antibacterials, Other	**Antifolate Antibacterials**
		Glycopeptide Antibacterials
		Lincomycin Antibacterials
		Nitrofuran Antibacterials
		Oxazolidinone Antibacterials
		Miscellaneous Antibacterials
Anticonvulsants	**Calcium Channel Modifying Agents**	
	Gamma-aminobutyric Acid (GABA) Augmenting Agents	
	Glutamate Reducing Agents	
	Sodium Channel Inhibitors	
	Anticonvulsants, Other	-
Antidementia Agents	**Cholinesterase Inhibitors**	
	Glutamate Pathway Modifiers	
	Antidementia Agents, Other	
Antidepressants	**Monoamine Oxidase Inhibitors**	
	Serotonin/Norepinephrine Reuptake Inhibitors	
	Tricyclics	-
	Antidepressants, Other	
Antidotes, Deterrents, and Toxicologic Agents	**Antidotes**	-
	Deterrents	**Alcohol Deterrents**
		Smoking Cessation Agents
	Toxicologic Agents	**Opioid Antagonists**
Antiemetics		**5-Hydroxytryptamine 3 (5-HT3) Antagonists**
		Neurokinin 1 (NK1)

		Receptor Antagonists
		Antiemetics, Other
Antifungals		**Allylamine Antifungals**
		Azole Antifungals
		Echinocandin Antifungals
		Polyene Antifungals
		Antifungals (Other)
Antigout Agents		**Renal Tubular Blocking Agents**
		Xanthine Oxidase Inhibitors
		Antigout Agents (Non-renal Tubular Blocking Agents and Non-xanthine Inhibitors)
Anti-inflammatory Agents	**Glucocorticoids**	
	Nonsteroidal Anti-inflammatory Drugs	-
Antimigraine Agents	**Abortive**	**Ergot Alkaloids**
		Triptans
	Prophylactic	**Beta-adrenergic Blocking Agents**
		Antimigraine Agents, Prophylactic (Non–beta-adrenergic Blocking Agents)
Antimyasthenic Agents	**Parasympathomimetics**	
Antimycobacterials	**Antituberculars**	
	Antimycobacterials, Other	
	Alkylating Agents	**Ethylenimines/ Methylmelamines**
	-	**Nitrogen Mustards**
	-	**Nitrosoureas**
	-	**Alkylating Agents, Other**
	Antiangiogenic Agents	-
	Antiestrogens/Modifiers	**Estrogen-nitrosoureas**
		Selective Estrogen Receptor Modulators, 1st Generation
		Purine Analogs and

Antineoplastics	**Antimetabolites**	**Related Inhibitors**
	-	**Antimetabolites, Other**
	Aromatase Inhibitors, 3rd Generation	
	Molecular Target Inhibitors	**Epidermal Growth Factor Receptor Kinase Inhibitors**
		Multitargeted Kinase Inhibitors, Bcr-Abl/c-kit Receptor Tyrosine Kinases
		Multitargeted Kinase Inhibitors, Vascular Endothelial Growth Factor Receptor Tyrosine Kinases
	Monoclonal Antibodies	**Anti-CD20 Antibodies**
	Retinoids	
	Antineoplastics, Other	-
Antiparasitics	**Anthelmintics**	
	Antiprotozoals	**Antimalarials**
		Antiprotozoals (Non-antimalarials)
	Pediculicides/ Scabicides	
Antiparkinson Agents		**Anticholinergics**
	-	**Catechol O-methyltransferase (COMT) Inhibitors**
	-	**Dopamine Agonists, Ergot**
		Dopamine Agonists, Nonergot
		Dopamine Precursors
		Monoamine Oxidase B (MAO-B) Inhibitors
	-	**Antiparkinson Agents, Other**
Antipsychotics	**Atypicals**	
	Conventional	
Antispasticity Agents		
	Anti-cytomegalovirus	

	(CMV) Agents	
	Antihepatitis Agents	
	Antiherpetic Agents	
	Anti-human Immunodeficiency Virus (HIV) Agents, Fusion Inhibitors	
Antivirals	Anti-HIV Agents, Non-nucleoside Reverse Transcriptase Inhibitors	
	Anti-HIV Agents, Nucleoside and Nucleotide Reverse Transcriptase Inhibitors	
	Anti-HIV Agents, Protease Inhibitors	
	Anti-influenza Agents	
Anxiolytics	Antidepressants	
	Anxiolytics, Other	
Bipolar Agents		
Blood Glucose Regulators	Antidiabetic Agents	Alpha Glucosidase Inhibitors
		Amylinomimetics
		Biguanides
		Dipeptidyl Peptidase-4 (DPP-4) Inhibitors
		Incretin Mimetics
		Meglitinides
		Sulfonylureas
		Thiazolidinediones
	Glycemic Agents	
	Insulins	Insulin, Rapid-acting
		Insulin, Short-acting
		Insulin, Intermediate-acting
		Insulin, Long-acting
		Insulin Mixtures, Short-acting and Intermediate-acting
		Insulin Mixtures, Analogs
	Anticoagulants	Anticoagulants, Oral
		Factor Xa Inhibitors,

		Indirect
		Low Molecular Weight Heparins
	Blood Formation Products	**Colony Stimulating Factors**
		Erythropoietins
Blood Products/Modifiers/Volume Expanders	**Coagulants**	**Protease Inhibitors**
	Platelet Aggregation Inhibitors	**Adenosine Diphosphate P2Y12 Inhibitors**
		Cyclic Adenosine Monophosphate Reuptake Inhibitors
		Phosphodiesterase III/Adenosine Uptake Inhibitors
	Alpha-adrenergic Agonists	
	Alpha-adrenergic Blocking Agents	
	Antiarrhythmics	**Antiarrhythmics - Classes IA, B, and C**
		Antiarrhythmics - Class Ia/II/III/IV
		Antiarrhythmics - Class II
		Antiarrhythmics - Class II/III
		Antiarrhythmics - Class III
		Antiarrhythmics - Class IV
	Beta-adrenergic Blocking Agents	**Alpha-beta–adrenergic Blocking Agents**
		Cardioselective Beta-adrenergic Blocking Agents
		Nonselective Beta-adrenergic Blocking Agents
	Calcium Channel	**Calcium Channel Blocking Agents (Non-**

Cardiovascular Agents	**Blocking Agents**	**dihydropyridines)**
		Dihydropyridines
	Diuretics	**Carbonic Anhydrase Inhibitors**
		Loop Diuretics
		Potassium-sparing Diuretics
		Thiazide Diuretics
	Dyslipidemics	**Bile Acid Sequestrants**
		Cholesterol Absorption Inhibitors
		Fibrates
		3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) Reductase Inhibitors
		Nicotinic Acid
		Omega-3 Fatty Acids
	Renin-angiotensin-aldosterone System Inhibitors	**Aldosterone Receptor Antagonists**
		Angiotensin-converting Enzyme (ACE) Inhibitors
		Angiotensin II Receptor Antagonists
	Vasodilators	**Vasodilators, Direct-acting Arterial**
		Vasodilators, Direct-acting Arterial/Venous
		Vasodilators, Endothelin Receptor Antagonists
	Cardiovascular Agents, Other	
Central Nervous System Agents	***Amphetamines, ADHD***	
	Non-amphetamines, ADHD	
	Non-amphetamines, Other	
Dental and Oral Agents		

Dermatological Agents		Dermatological Acne Agents
		Dermatological Anti-inflammatory Agents
		Dermatological Antipruritic Agents
		Dermatological Calcineurin Inhibitors
		Dermatological Caustic Agents
		Dermatological Emollients
		Dermatological Genital Wart Agents
		Dermatological Mitotic Inhibitors
		Dermatological Non-melanoma Skin Cancer Agents
		Dermatological Photochemotherapy Agents
		Dermatological Psoriasis Agents
		Dermatological Wound Care Agents
Enzyme Replacements/Modifiers		Anti-cystine Agents
		Fabry Disease Treatment
		Gaucher's Disease Treatment
		Glucosylceramide Synthase Inhibitors
		Hereditary Tyrosinemia Type 1 (HT-1) Treatment
		Homocystinuria Treatment
		Hunter Syndrome Treatment
		Mucopolysaccharidosis Disease Treatment
		Pancrelipase

		Replacement
		Severe Combined Immunodeficiency Disease (SCID) Treatment
		Sucrase Enzyme Replacement
		Urea Cycle Disorder Treatment
Gastrointestinal Agents	***Antispasmodics, Gastrointestinal***	
	Histamine$_2$ (H_2) Blocking Agents	
	Irritable Bowel Syndrome Agents	
	Protectants	
	Proton Pump Inhibitors	
	Gastrointestinal Agents, Other	
Genitourinary Agents	**Antispasmodics, Urinary**	
	Benign Prostatic Hypertrophy Agents	**Alpha 1-adrenergic Blocking Agents**
		5 Alpha-reductase Inhibitors
	Phosphate Binders	
	Genitourinary Agents, Other	
Hormonal Agents, Stimulant/ Replacement/ Modifying (Adrenal)	**Glucocorticoids/ Mineralocorticoids**	**Glucocorticoids-Systemic**
		Glucocorticoids-Topical-Low Potency
		Glucocorticoids-Topical-Medium Potency
		Glucocorticoids-Topical-High Potency
		Glucocorticoids-Topical-Very High Potency
	-	**Mineralocorticoids**
Hormonal Agents,	-	**Gonadotropins**
		Growth Hormone

Stimulant/ Replacement/ Modifying (Pituitary)		**Analogs**
		Insulin-like Growth Factor Analogs
		Vasopressin Analogs
Hormonal Agents, Stimulant/ Replacement/ Modifying (Prostaglandins)	-	
Hormonal Agents, Stimulant/ Replacement/ Modifying (Sex Hormones/ Modifiers)	**Anabolic Steroids**	-
	Androgens	
	Estrogens	-
	Progestins	-
	Selective Estrogen Receptor Modifying Agents	-
Hormonal Agents, Stimulant/ Replacement/ Modifying (Thyroid)	-	
Hormonal Agents, Suppressant (Adrenal)	-	
Hormonal Agents, Suppressant (Parathyroid)	-	**Calcimimetics**
Hormonal Agents, Suppressant (Pituitary)	-	**Dopamine Agonists**
	-	**Gonadotropin-releasing Hormone Analogs**
		Growth Hormone Antagonists
	-	**Somatostatin Analogs**
Hormonal Agents, Suppressant (Sex Hormones/ Modifiers)	**Antiandrogens**	-
Hormonal Agents, Suppressant (Thyroid)	**Antithyroid Agents**	
	Immune Stimulants	**Vaccines to Prevent Anthrax**
		Vaccines to Prevent Diphtheria
		Vaccines to Prevent Haemophilus Type B
		Vaccines to Prevent

		Hepatitis A
		Vaccines to Prevent Hepatitis B
		Vaccines to Prevent Japanese Encephalitis
		Vaccines to Prevent Measles
		Vaccines to Prevent Meningococcal Disease
		Vaccines to Prevent Mumps
		Vaccines to Prevent Papillomavirus Disease
		Vaccines to Prevent Pertussis
		Vaccines to Prevent Poliovirus
Immunological Agents		Vaccines to Prevent Rabies
		Vaccines to Prevent Rotavirus Disease
		Vaccines to Prevent Rubella
		Vaccines to Prevent Tetanus
		Vaccines to Prevent Typhoid
		Vaccines to Prevent Varicella
		Vaccines to Prevent Yellow Fever
		Vaccines to Prevent Zoster
	Immune Suppressants	Immune Suppressants (Non-TNF Inhibitors)
		Tumor Necrosis Factor (TNF) Inhibitors
	Immunizing Agents, Passive	Immunoglobulins
	Immunomodulators	Interferons, Alfa
		Interferons, Beta
		Interferons, Gamma

		Immunomodulators, Other
Inflammatory Bowel Disease Agents	**Glucocorticoids**	
	Salicylates	
	Sulfonamides	
Metabolic Bone Disease Agents		**Bisphosphonates, Oral**
		Bisphosphonates, Parenteral
		Calcium Regulating Hormones
		Parathyroid Hormone Analogs
		Vitamin D–related Agents/Metabolic Bone Disease Agents
Ophthalmic Agents	**Ophthalmic Anti-allergy Agents**	
	Ophthalmic Antiglaucoma Agents	**Alpha-adrenergic Agonists, Ophthalmic**
		Beta-adrenergic Blocking Agents, Ophthalmic
		Carbonic Anhydrase Inhibitors, Ophthalmic
		Cholinergic Agonists, Ophthalmic
	Ophthalmic Anti-inflammatories	**Glucocorticoids, Ophthalmic**
		Nonsteroidal Anti-inflammatory Drugs, Ophthalmic
	Ophthalmic Prostaglandin and Prostamide Analogs	
	Ophthalmic Agents, Other	-
Otic Agents	-	**Otic Anti-inflammatories**
	-	
	Antihistamines	**Histamine$_1$ (H_1) Blocking Agents, Mildly/Non-sedating**
		H_1 Blocking Agents, Sedating

Respiratory Tract Agents	**Anti-inflammatories, Inhaled Corticosteroids**	
	Antileukotrienes	**Receptor Antagonists**
		Synthesis Inhibitors
	Bronchodilators, Anticholinergic	
	Bronchodilators, Phosphodiesterase Inhibitors (Xanthines)	
	Bronchodilators, Sympathomimetic	
	Mast Cell Stabilizers	
	Pulmonary Antihypertensives	
	Respiratory Tract Agents, Other	
Sedatives/Hypnotics		
Skeletal Muscle Relaxants		
Therapeutic Nutrients/Minerals/ Electrolytes	**Electrolytes/Minerals**	
	Vitamins	**Prenatal Vitamins**

EXHIBIT 389

Transfer of Therapeutic Biological Products to the Center for Drug Evaluation and Research

On June 30, 2003, FDA transferred some of the therapeutic biological products that had been reviewed and regulated by the Center for Biologics Evaluation and Research (CBER) to the Center for Drug Evaluation and Research (CDER). CDER now has regulatory responsibility, including premarket review and continuing oversight, over the transferred products. In regulating the products assigned to them, CBER and CDER will consult with each other regularly and whenever necessary. On October 1, 2003, the staff comprising CBER's Office of Therapeutics Research and Review also transferred to CDER.

The lists below identify categories of biological products transferred from CBER to CDER, and categories of biological products remaining in CBER. Please note that the CBER list contains only a portion of the products CBER currently regulates; this list contains products that are closely related in chemical structure to products that transferred to CDER, e.g. therapeutic proteins and polysaccharides. Products are included on the CBER list as a means of clarifying the products that transferred and those that did not.

Categories of Biological Products Transferred to CDER

- Monoclonal antibodies for in vivo use.
- Proteins intended for therapeutic use, including cytokines (e.g. interferons), enzymes (e.g. thrombolytics), and other novel proteins, except for

those that are specifically assigned to CBER (e.g., vaccines and blood products). This category includes therapeutic proteins derived from plants, animals, or microorganisms, and recombinant versions of these products.

- Immunomodulators: proteins or peptides that are not antigen specific (e.g., cytokines, growth factors, chemokines, etc.) that are intended to treat disease by inhibiting or modifying a pre-exisiting immune response; and proteins or peptides intended to act in antigen-specific fashion to treat or prevent autoimmune diseases by inhibiting or modifying pre-existing immune responses.
- Growth factors, cytokines, and monoclonal antibodies intended to mobilize, stimulate, decrease or otherwise alter the production of cells in vivo.[1] This category includes growth factors, cytokines, and monoclonal antibodies, as well as non-biological agents, administered as mobilizing agents for their direct therapeutic effect on the recipient, as well as growth factors, cytokines, and monoclonal antibodies administered for the purpose of subsequently harvesting the mobilized, stimulated, decreased or otherwise altered cells for use in a human cellular or tissue-based product (HCT/P).

Categories of Biological Products Remaining in CBER

- Cellular products, including products composed of human, bacterial or animal cells (such as pancreatic islet cells for transplantation), or from physical parts of those cells (such as whole cells, cell fragments, or other components intended for use as preventative or therapeutic vaccines).

- Gene therapy products. Human gene therapy/gene transfer is the administration of nucleic acids, viruses, or genetically engineered microorganisms that mediate their effect by transcription and/or translation of the transferred genetic material, and/or by integrating into the host genome. Cells may be modified in these ways ex vivo for subsequent administration to the recipient, or altered in vivo by gene therapy products administered directly to the recipient.
- Vaccines and vaccine-associated products: products, regardless of their composition or method of manufacture, intended to induce or enhance a specific immune response to prevent or treat a disease or condition, or to enhance the activity of other therapeutic interventions.
- Allergenic extracts used for the diagnosis and treatment of allergic diseases and allergen patch tests.
- Antitoxins, antivenins, and venoms
- Blood, blood components, plasma derived products (for example, albumin, immunoglobulins, clotting factors, fibrin sealants, proteinase inhibitors), including recombinant and transgenic versions of plasma derivatives, (for example clotting factors), blood substitutes, plasma volume expanders, human or animal polyclonal antibody preparations including radiolabeled or conjugated forms, and certain fibrinolytics such as plasma-derived plasmin, and red cell reagents.
- Human cells, tissues and cellular and tissue-based products (HCT/P's). This category includes HCT/P's containing cells that have been harvested following in vivo administration of a CDER-regulated growth factor, cytokine, or monoclonal antibody,[2] as well as HCT/P's requiring ex vivo manipulation.

Combination Products

The lists above contain some combination products comprised of a biological product component with a device and/or drug component, though such products are not specifically identified. Combination products are assigned to a Center for review and regulation in accordance with the products' primary mode of action.[3] When a product's primary mode of action is attributable to a type of biological product assigned to CDER, the product will be assigned to CDER. Similarly, when a product's primary mode of action is attributable to a type of biological product assigned to CBER, the product will be assigned to CBER. For further information about combination products, see the Combination Products (/combination-products) section of the FDA website, or contact the Office of Combination Products at 301-796-8930, or combination@fda.gov.

Further Information

Questions about the assignment of specific products to CBER or CDER should be directed to the center jurisdiction officers at:

CDER Ombudsman 301-796-3436

CBER Ombudsman 301-827-0379

Footnotes

[1] CBER reviews and regulates some products other than growth factors, cytokines, and monoclonal antibodies that are mobilizing agents in that they are administered in vivo for mobilizing, stimulating, decreasing or otherwise altering the production or function of cells or tissues that are subsequently harvested for use in an HCT/P. The mobilizing

agents and other cell manipulating agents reviewed and regulated by CBER also fall into one of the categories of products currently assigned to CBER (e.g., a vaccine or gene therapy).

[2] The most efficient way to investigate an HCT/P developed from cells that have been harvested following in vivo administration of a growth factor, cytokine, or monoclonal antibody would ordinarily be to first investigate the safety and activity of the growth factor, cytokine, or monoclonal antibody in mobilizing, stimulating, decreasing or otherwise altering cells in vivo, and then to reference this information in a subsequent application to CBER for the HCT/P. The Center jurisdiction officers listed below are available to discuss the various options and appropriate regulatory approaches.

[3] See 21 U.S.C. § 353(g), section 503(g) of the Federal Food, Drug, and Cosmetic Act.

EXHIBIT 390

IRB Waiver or Alteration of Informed Consent for Clinical Investigations Involving No More Than Minimal Risk to Human Subjects

Guidance for Sponsors, Investigators, and Institutional Review Boards

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(3) without initially seeking prior comment. The Agency has determined that prior public participation is not feasible or appropriate because this guidance presents a less burdensome policy that is consistent with the public health. Although this guidance document is immediately in effect, it remains subject to public comment in accordance with the Agency's good guidance practices regulation (21 CFR 10.115).

You may submit comments or suggestions at any time. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this document, contact Janet Norden, 301-796-1127; Carol Drew, 301-796-8510; (CDER) Ebla Ali Ibrahim, Office of Medical Policy, 301-796-3691; (CBER) Office of Communication, Outreach and Development, 800-835-4709 or 240-402-8010; or (CDRH) Office of Device Evaluation, Clinical Trials Program, 301-796-5640.

U.S. Department of Health and Human Services
Food and Drug Administration
Office of Good Clinical Practice (OGCP)
Center for Drug Evaluation and Research (CDER)
Center of Biologics Evaluation and Research (CBER)
Center for Devices and Radiological Health (CDRH)

July 2017

IRB Waiver or Alteration of Informed Consent for Clinical Investigations Involving No More Than Minimal Risk to Human Subjects

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Guidance for Sponsors, Investigators, and Institutional Review Boards

Additional copies are available from:

Office of Good Clinical Practice
Office of Special Medical Programs, Office of Medical Products and Tobacco
Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993
(Tel) 301-796-8340
http://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/GuidancesInformationSheetsandNotices/ucm219433.htm

U.S. Department of Health and Human Services
Food and Drug Administration

July 2017

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IRB Waiver or Alteration of Informed Consent for Clinical Investigations Involving No More than Minimal Risk to Human Subjects

Guidance for Sponsors, Investigators, and IRBs[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This document provides guidance to sponsors, investigators, and institutional review boards (IRBs) on enforcement of FDA regulations governing informed consent requirements for clinical investigations that involve no more than minimal risk[2] to human subjects. This guidance informs sponsors, investigators, IRBs and other interested parties that the FDA does not intend to object to an IRB waiving or altering informed consent requirements for certain minimal risk clinical investigations as described in Section IV of this guidance. In addition, FDA does not intend to object to a sponsor initiating, or an investigator conducting, a minimal risk clinical investigation for which an IRB waives or alters the informed consent requirements as described in Section IV of this guidance.

Over the years, FDA has received numerous inquiries from sponsors and investigators about conducting important minimal risk clinical investigations for which obtaining informed consent was not practicable. Many of these minimal risk clinical investigations did not proceed because FDA did not have the statutory authority to permit a waiver of informed consent for such investigations. As described in Section II of this document, an amendment to the Federal Food, Drug and Cosmetic Act (FD&C Act) has provided FDA with authority to permit an exception from informed consent for minimal risk clinical investigations when specific criteria are met. Since this amendment passed, FDA has received additional questions regarding requirements for informed consent in minimal risk clinical investigations. FDA believes this guidance will facilitate the conduct of certain minimal risk clinical investigations that are important to addressing significant public health needs without compromising the rights, safety, or welfare of

[1] This guidance has been prepared by the Office of Good Clinical Practice, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research and the Center for Devices and Radiological Health at the Food and Drug Administration.

[2] Minimal risk is defined in applicable FDA regulations as "the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." (21 CFR 50.3(k), 56.102(i)).

human subjects. Although this guidance is immediately in effect, FDA will consider all comments received and will revise this guidance when appropriate.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

On December 13, 2016, the 21st Century Cures Act (Cures Act) (P.L. 114-255) was signed into law. Title III, section 3024 of the Cures Act amended sections 520(g)(3) and 505(i)(4) of the FD&C Act to provide FDA with the authority to permit an exception from informed consent requirements when the proposed clinical testing poses no more than minimal risk to the human subject and includes appropriate safeguards to protect the rights, safety, and welfare of the human subject. This statutory amendment became effective on December 13, 2016. FDA intends to promulgate regulations to reflect this statutory change, including appropriate human subject protection safeguards.

Currently, FDA's regulations governing the protection of human subjects (21 CFR parts 50 and 56) allow exception from the general requirements for informed consent only in life-threatening situations when certain conditions are met (21 CFR 50.23) or when the requirements for emergency research are met (21 CFR 50.24). This limitation in FDA's regulations stemmed from section 520(g)(3)(D) of the FD&C Act, relating to the investigational use of devices. Before the Cures Act amendments, this provision in the FD&C Act directed that FDA regulations require informed consent be obtained except where the investigator "determines in writing that there exists a life threatening situation involving the human subject of such testing which necessitates the use of such device" and it is not feasible to get the consent of the subject or the subject's representative.

The requirement in section 505(i) of the FD&C Act for informed consent for investigational use of drugs (including biologics) provided that FDA regulations must ensure informed consent is obtained "except where it is not feasible or it is contrary to the best interest of such human beings." In order to promote consistency across medical products, FDA adopted regulations reflecting the device standard for all medical product research.

In general, FDA's regulations governing the protection of human subjects conform to the requirements in the "Federal Policy for the Protection of Human Subjects" (the Common Rule), with a few exceptions because of differences in FDA's mission or statutory authority. The Common Rule, originally promulgated in 1991[3], sets forth requirements for the protection of

[3] The Common Rule was recently revised to better protect human subjects involved in research, facilitate valuable research, and reduce burden, delay and ambiguity for investigators (82 FR 7149, January 19, 2017). The final rule that revised the Common Rule adopts an effective and general compliance date of January 19, 2018. References to the Common Rule in this document are to the pre-2018 requirements that are in effect at the time of issuance of this guidance.

human subjects involved in research that is conducted or supported by the Department of Health and Human Services (HHS) (see 45 CFR 46, Subpart A) and 15 other Federal departments and agencies. The purpose of the Common Rule is to promote uniformity, understanding, and compliance with human subject protections as well as to create a uniform body of regulations across the Federal departments and agencies.[4] FDA regulations and the Common Rule share the same definition for "minimal risk," but the Common Rule allows a waiver of informed consent for minimal risk research if specific criteria are met. As stated above, FDA's regulations currently do not include an exception from informed consent for minimal risk clinical investigations.[5]

III. DISCUSSION

The Common Rule standard has been adopted and successfully employed for decades by numerous other Federal agencies. The Common Rule permits an IRB to waive the requirements to obtain informed consent, or to allow changes to, or omission of, some or all elements of informed consent if the IRB finds and documents that: (1) the research involves no more than minimal risk to the subjects; (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects; (3) the research could not practicably be carried out without the waiver or alteration; and (4) whenever appropriate, the subjects will be provided with additional pertinent information after participation. (45 CFR 46.116(d)).[6]

The Secretary's Advisory Committee on Human Research Protections (SACHRP) provided input on the issue of whether waiver of informed consent provisions for certain minimal risk clinical investigations would be appropriate and helpful to FDA-regulated research. On March 13, 2014, SACHRP considered this issue. Recognizing that harmonization with the Common Rule would promote consistency and help to reduce confusion in the research community about when a waiver of informed consent may be permitted, while also facilitating certain FDA-regulated research, SACHRP recommended to the Secretary of HHS that FDA adopt the provisions for waiver of informed consent that exist under the Common Rule at 45 CRF 46.116(d). On October 26, 2016, SACHRP reiterated that recommendation to the Secretary.[7]

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[4] 80 FR 53931 at 53935, September 8, 2015.

[5] Note that this exception from the requirement to obtain informed consent differs from the waiver from the requirement for documentation of informed consent permitted under both the Common Rule and FDA regulations (45 CFR 46.117(c); 21 CFR 56.109(c)).

[6] The final rule that recently revised the Common Rule (82 FR 7149, January 19, 2017) adds a fifth criterion (i.e., "if the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format" (new 2018 requirement at 45 CFR 46.116(f)(3)(iii)). As FDA revises its regulations to harmonize to the extent appropriate and permissible with the Common Rule, we will consider including this new criterion in any waiver provision.

[7] SACHRP's recommendations are available at https://www.hhs.gov/ohrp/sachrp-committee/recommendations/2014-july-3-letter-attachment-c/index.html and https://www.hhs.gov/ohrp/sachrp-committee/recommendations/attachment-b-november-2-2016-letter/index.html.

IV. IRB WAIVER OR ALTERATION OF INFORMED CONSENT

Waiver of informed consent for certain FDA-regulated minimal risk clinical investigations will facilitate investigators' ability to conduct studies that may contribute substantially to the development of products to diagnose or treat diseases or conditions, or address unmet medical needs. In light of the Cures Act amendment to the FD&C Act described above, FDA intends to revise its informed consent regulations to add this waiver or alteration under appropriate human subject protection safeguards to the two existing exceptions from informed consent (i.e., in life-threatening situations and for emergency research). However, until FDA promulgates these regulations, we do not intend to object to an IRB[8] approving a consent procedure that does not include, or that alters, some or all of the elements of informed consent set forth in 21 CFR 50.25, or waiving the requirements to obtain informed consent when the IRB finds and documents[9] that:

1. The clinical investigation involves no more than minimal risk (as defined in 21 CFR 50.3(k) or 56.102(i)) to the subjects;
2. The waiver or alteration will not adversely affect the rights and welfare of the subjects;
3. The clinical investigation could not practicably be carried out without the waiver or alteration; and
4. Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

FDA does not intend to object to a sponsor initiating, or an investigator conducting, a minimal risk clinical investigation for which an IRB waives or alters the informed consent requirements as described above. FDA intends to withdraw this guidance after we promulgate regulations to permit a waiver or alteration of informed consent under appropriate human subject protection safeguards consistent with section 3024 of the Cures Act.

V. INQUIRIES ABOUT SPECIFIC CLINICAL INVESTIGATIONS

Sponsors, investigators and IRBs may contact FDA for questions about implementing the recommendations in this guidance for a specific clinical investigation. Questions should be directed to the appropriate Center contact listed below.

Center for Drug Evaluation and Research
Ebla Ali Ibrahim
Office of Medical Policy Initiatives, Office of Medical Policy
301-796-2500 or 301-796-3691
Email: Ebla.Ali-Ibrahim@fda.hhs.gov

[8] An institutional review board (IRB) is defined in 21 CFR 56.102(g) and is subject to the requirements of 21 CFR part 56.

[9] An IRB is required to prepare and maintain adequate documentation of its activities, including actions taken by the IRB, under 21 CFR 56.115.

Center for Biologics Evaluation and Research
Office of Communication, Outreach and Development
800-835-4709 or 240-402-8010
Email: ocod@fda.hhs.gov

Center for Devices and Radiological Health
Office of Device Evaluation, Office of the Director
Clinical Trials Program
301-796-5640
Email: CDRHClinicalEvidence@fda.hhs.gov

EXHIBIT 391

Protection of Human Subjects; Informed Consent

21 CFR Parts 50, 71, 171, 180, 310, 312, 314, 320, 330, 361, 430, 431, 601, 630, 812, 813, 1003, 1010

[Docket No. 78N-0400]

46 FR 8942

January 27, 1981

AGENCY: Food and Drug Administration.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing regulations to provide protection for human subjects of clinical investigations conducted pursuant to requirements for prior submission to FDA or conducted in support of applications for permission to conduct further research or to market regulated products. The regulations clarify existing FDA requirements governing informed consent and provide protection of the rights and welfare of human subjects involved in research activities that fall within FDA's jurisdiction.

EFFECTIVE DATE: July 27, 1981.

FOR FURTHER INFORMATION CONTACT: John C. Petricciani, Office of the Commissioner (HFB-4), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20205, 301-496-9320.

TEXT: SUPPLEMENTARY INFORMATION:

argued that the concept of informed consent had not changed since the Drug Amendments were enacted in 1962, and neither comment offered any particular investigational situation in which they thought an investigator might reasonably determine, as provided in sections 505(i) and 507(d) of the act, that obtaining informed consent would not be "feasible" or "in an investigator's professional judgment, [would be] contrary to [a subject's] best interests."

Only one of the comments objecting to the promulgation of a single standard offered any extensive rationale for the objection raised. This comment argued that FDA should perpetuate in its informed consent regulation, the "therapeutic privilege" exemption provided by Congress when it enacted the 1962 Drug Amendments. This comment stated that in choosing to disregard the "therapeutic privilege" exemption, FDA was intruding into both the realm of congressional prerogative and the practice of medicine.

According to this comment, the circumstances in which the "therapeutic privilege" ought to apply, were as follows:

* * * A departure from the absolute requirement of informed consent is necessitated when "patient psychology" is such that a physician must be free to use a new therapeutic measure, without obtaining the patient's informed consent, if in his judgment it offers help of saving life, re-establishing health, or alleviating suffering. When a drug is being used in a clinical investigation *primarily for treatment,* the circumstances call forth the standards pertinent to the traditional physician-patient relationship, instead of those applicable to pure research. (Emphasis added.)

Basically, this comment assumes that a clinical investigation which involves an investigational article used primarily for treatment is not really an "investigation" at all, but is simply "the practice of medicine," and the basic objection expressed

seems to be that obtaining informed consent could unjustifiably frighten patients away from participation in an investigational study that might provide significant benefits for that individual and/or society as a whole, while presenting little or no risk to the individual participant.

FDA has considered the objections raised by these comments, has conducted an extensive review of the current legal requirements for informed consent in the treatment as opposed to the investigational/experimental setting, and finds, for the reasons discussed below, that the uniform approach proposed is justified.

The "therapeutic privilege" in the context of experimentation has been subject to increased criticism in recent years. In a paper on the Law of Informed Consent prepared for the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (Ref. *1*), the authors concluded that nondisclosure based upon a physician's judgment that it is not in the patient's best interest to know, should never be allowed in the experimental setting.

The authors of this report, who surveyed international, Federal, and local standards of informed consent, concluded that because the purpose of the "therapeutic privilege" doctrine was to make sure that patients get treatment that physicians believe they need, it could have no application to nontherapeutic experimentation where no treatment is involved. The authors also concluded that,

* * * Because of the great potential for abuse, e.g., the withholding of information for convenience or to assure the patient will not reject the treatment, and because the probability of success with an experimental treatment is either not known or very low, this exception should also not be permitted in the case of therapeutic experimentation. Indeed, as has been noted by a number of commentators, in

this situation the physician-experimenter may have much more ability to obtain consent for an experiment than he would have from a normal volunteer who neither has an established dependency relation with him nor expects that the proposed experiment might be personally beneficial to him. As Professor Alexander Capron has observed: The "normal volunteer" solicited for an experiment is in a good position to consider the physical, psychological, and monetary risks and benefits to him when he consents to participate. How much harder that is for the patient to whom an experimental technique is offered during a course of treatment! The man proposing the experiment is one to whom the patient may be deeply indebted for past care (emotionally as well as financially) and on whom he is probably dependent for his future well-being. The procedure may be offered, despite unknown risks, because more conventional methods have proved ineffective. Even when a successful but slow recovery is being made, patients offered new therapy often have eyes only for its novelty and not for the risks.

In order to protect self-determination and promote rational decision-making, more, not less, information should probably be required to be disclosed in the experimental therapy situation than in the purely experimental setting with a normal volunteer (Ref. *1*).

FDA agrees with the findings contained in the special report on the Law of Informed Consent. The standard of practice regarding informed consent promulgated by Congress in the Drug Amendments of 1962 was the standard that prevailed at that time. It is not the standard of practice today. FDA is concerned that research subjects be adequately protected from abuses of the kind that have taken place in the past (44 FR 47713-17); and is convinced that one way to protect

research subjects against abuse is to ensure that they have the opportunity to be adequately informed before they consent to participate.

FDA does not believe that promulgating a single standard that reflects both current congressional thinking and current standards regarding the practice of medicine represents an unreasonable encroachment upon the prerogatives of either Congress or the medical community. Congress expressly recognized at the time the Medical Device Amendments of 1976 were passed that, in view of changing social policy and advancing biomedical technology, the informed consent provisions of the Medical Device Amendments should be implemented through regulations based upon the recommendations to be made by the National Commission (Ref. *2*). Indeed, the very purpose for which Congress established the National Commission was to assure a thorough review of the basic ethical principles underlying the conduct of biomedical and behavioral research (44 FR 47716).

FDA believes that the regulation does not encroach upon the prerogatives of the medical community because a review of court decisions which have involved informed consent casts doubt on whether the so-called "therapeutic privilege" to dispense with informed consent has any continued viability even in the standard practice of medicine. With increasing frequency, courts have held that when a patient is harmed by a treatment to which he or she might not have consented had he or she been adequately informed of the risks involved in that treatment, the doctor's failure to obtain informed consent may result in a finding of liability for negligence. In *Cobbs* v. *Grant,* 8 Cal. 3d 229, 502 P.2d 1 (1972), the California Supreme Court discussed at length the thesis that medical doctors are invested with discretion to withhold information from their patients and found that discretion to

be extremely limited, stating that, "it is the prerogative of the patient, not the physician, to determine for himself the direction in which he believes his interests lie. To enable the patient to chart his course knowledgeably, reasonable familiarity with the therapeutic alternatives and their hazards becomes essential." *Cobbs, supra,* at 242-243. The California Court held that a duty of reasonable disclosure of the available choices with respect to proposed therapy and of the dangers inherently and potentially involved in each choice was an "integral part of the physician's overall obligation to the patient." *Cobbs, supra,* at 243. Under the *Cobbs* rationale, a patient's informed consent is an absolute requirement except in an emergency situation or in a situation in which the patient is a child or incompetent, in which case consent is either implied or sought from a legal guardian. Thus, in *Cobbs,* the California Court found that consent of the quality required by this regulation should have been obtained from the patient and that it was the patient's prerogative to make the treatment decision based upon adequate information, not the physician's prerogative to limit the patient's choices by limiting the information provided. See generally, *Pharmaceutical Manufacturers* v. *Food Drug Administration,* 484 F. Supp. 1179, 1188 (D. Del. 1980).

The subject of negligence and informed consent is also discussed at length in *Canterbury* v. *Spence,* 464 F.2d 772 (D.C. Cir. 1972), *cert. denied,* 409 U.S. 1064 (1972), an action involving, among other things, the sufficiency of the information provided to a patient. Beginning with the fundamental premise that, "every human being of adult years and sound mind has a right to determine what shall be done with his own body," the *Canterbury* court defines "true consent" as the informed exercise of a choice that, in turn, entails an opportunity to evaluate knowledgeably the options available and the risks attendant upon each. *Canterbury,*

EXHIBIT 392

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

INFORMED CONSENT ACTION NETWORK,

Plaintiff,

-against-

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

Defendant.

STIPULATION

18-cv-03215 (JMF)

WHEREAS, 42 U.S.C. § 300aa-27, entitled "Mandate for safer childhood vaccines," provides as follows:

> (a) General rule
>
> In the administration of this part and other pertinent laws under the jurisdiction of the Secretary [of the Department of Health and Human Services], the Secretary shall—
>
> > (1) promote the development of childhood vaccines that result in fewer and less serious adverse reactions than those vaccines on the market on December 22, 1987, and promote the refinement of such vaccines, and
> >
> > (2) make or assure improvements in, and otherwise use the authorities of the Secretary with respect to, the licensing, manufacturing, processing, testing, labeling, warning, use instructions, distribution, storage, administration, field surveillance, adverse reaction reporting, and recall of reactogenic lots or batches, of vaccines, and research on vaccines, in order to reduce the risks of adverse reactions to vaccines.
>
> . . .
>
> (c) Report
>
> Within 2 years after December 22, 1987, and periodically thereafter, the Secretary shall prepare and transmit to the Committee on Energy and Commerce of the House of Representatives and the Committee on Labor and Human Resources of the Senate a report describing the

Exhibit 392

> actions taken pursuant to subsection (a) of this section during the preceding 2-year period.

WHEREAS, on August 25, 2017, Informed Consent Action Network ("ICAN") submitted a Freedom of Information Act request (the "FOIA Request") to the Department of Health and Human Services ("HHS" or the "Department"), which was assigned control number 2017-01119-FOIA-OS, that sought the following records:

> **Any and all reports transmitted to the Committee on Energy and Commerce of the House of Representatives and the Committee on Labor and Human Resources of the Senate by the Secretary of HHS pursuant to 42 U.S.C. §300aa-27(c).**

WHEREAS, on April 12, 2018, ICAN filed a Complaint for Declaratory and Injunctive Relief in the United States District Court, Southern District of New York against HHS seeking records, if any, responsive to the FOIA Request;

WHEREAS, the HHS Immediate Office of the Secretary ("IOS") maintains the official correspondence file of the Secretary of HHS, including reports to Congress by the Secretary of HHS, and therefore those files were most likely to contain records responsive to the FOIA Request;

WHEREAS, on June 27, 2018, HHS sent ICAN the following response to the FOIA Request:

> The [Department]'s searches for records did not locate any records responsive to your request. The Department of Health and Human Services (HHS) Immediate Office of the Secretary (IOS) conducted a thorough search of its document tracking systems. The Department also conducted a comprehensive review of all relevant indexes of HHS Secretarial Correspondence records maintained at Federal Records Centers that remain in the custody of HHS. These searches did not locate records responsive to your request, or indications that records responsive to your request and in the custody of HHS are located at Federal Records Centers.

WHEREAS, ICAN believes the foregoing response from HHS now resolves all claims asserted in this action;

IT IS HEREBY STIPULATED AND AGREED, by and between the parties by and through their respective counsel:

1. That the above-captioned action is voluntarily dismissed, with prejudice, pursuant to Federal Rule of Civil Procedure 41(a)(1)(A)(ii), each side to bear its own costs, attorney fees, and expenses; and

2. That this stipulation may be signed in counterparts, and that electronic (PDF) signatures may be deemed originals for all purposes.

Dated: July 6, 2018
New York, New York

KENNEDY & MODONNA LLP
Attorney for Plaintiff

By: _____
Robert F. Kennedy, Jr.
48 Dewitt Mills Road
Hurley, NY 12443
(845) 481-2622

Dated: July 6, 2018
New York, New York

GEOFFREY S. BERMAN
United States Attorney
Attorney for Defendant

By: _____
ANTHONY J. SUN
Assistant United States Attorney
86 Chambers Street, Third Floor
New York, New York 10007
(212) 637-2810
anthony.sun@usdoj.gov

SO ORDERED:

_____
HON. JESSE M. FURMAN, U.S.D.J.

Dated: New York, New York
July 6, 2018

Any pending motions are moot. All conferences are vacated. The Clerk of Court is directed to close the case.

Informed Consent Action Network

For Immediate Release: July 13, 2018

US District Court Judge signs order granting Plaintiff, Informed Consent Action Network (ICAN) and counsel, Robert F. Kennedy, Jr., the relief sought in a lawsuit against the US Department of Health and Human Services (HHS)

On Monday, June 9th, the United States District Court for the Southern District of New York signed an order granting Plaintiff, the nonprofit Informed Consent Action Network (ICAN), the relief it sought against the Defendant, the United States Department of Health and Human Services, HHS. ICAN was represented by Robert F. Kennedy, Jr.

In May 2017, ICAN Founder, Del Bigtree, Robert F. Kennedy, Jr.. and a handful of other individuals concerned about vaccine safety were selected by the White House to participate in a seminal meeting with the Counselor to the Secretary of HHS, the heads of the National Institute of Health, NIH, the Center for Disease Control, CDC, and Food and the Drug Administration, FDA. Del Bigtree and Robert F. Kennedy, Jr. suspected that HHS was not fulfilling its critical vaccine safety obligations as required by Congress in The National Childhood Vaccine Injury Act of 1986.

The 1986 Act granted unprecedented, economic immunity to pharmaceutical companies for injuries caused by their products and eviscerated economic incentive for them to manufacture safe vaccine products or improve the safety of existing vaccine products. Congress therefore charged the Secretary of HHS with the explicit responsibility to assure vaccine safety.

Hence, since 1986, HHS has had the primary and virtually sole responsibility to make and assure improvements in the licensing, manufacturing, adverse reaction reporting, research, safety and efficacy testing of vaccines in order to reduce the risk of adverse vaccine reactions. In order to assure HHS meets its vaccine safety obligations, Congress required as part of the 1986 Act that the Secretary of HHS submit a biennial reports to Congress detailing the improvements in vaccine safety made by HHS in the preceding two years.

ICAN therefore filed a Freedom of Information Act, FOIA, request on August 25th, 2017 to HHS seeking copies of the biennial reports that HHS was supposed to submit to Congress, starting in 1988, detailing the improvements it made every two years to vaccine safety. HHS stonewalled ICAN for eight months refusing to provide any substantive response to this request.

Informed Consent Action Network

ICAN was therefore forced to file a lawsuit to force HHS to either provide copies of its biennial vaccine safety reports to Congress or admit it never filed these reports. The result of the lawsuit is that HHS had to finally and shockingly admit that it never, not even once, submitted a single biennial report to Congress detailing the improvements in vaccine safety. This speaks volumes to the seriousness by which vaccine safety is treated at HHS and heightens the concern that HHS doesn't have a clue as to the actual safety profile of the now 29 doses, and growing, of vaccines given by one year of age.

In contrast, HHS takes the other portions of the 1986 Act, which require promoting vaccine uptake, very seriously, spending billions annually and generating a steady stream of reports on how to improve vaccine uptake. Regrettably, HHS has chosen to focus on its obligation to increase vaccine uptake and defend against any claim vaccines cause harm in the National Injury Vaccine Compensation Program (aka, the Vaccine Court) to such a degree that it has abandoned its vaccine safety responsibilities. If HHS is not, as confirmed in Court this week, even fulfilling the simple task of filing a biennial report on vaccine safety improvements, there is little hope that HHS is actually tackling the much harder job of actually improving vaccine safety.

For additional information or interviews please contact:
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EXHIBIT 393

COVID-19 Update

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Study Suggests Medical Errors Now Third Leading Cause of Death in the U.S. - 05/03/2016

Study Suggests Medical Errors Now Third Leading Cause of Death in the U.S.

Physicians advocate for changes in how deaths are reported to better reflect reality
Release Date: May 3, 2016

Share Fast Facts

- 10 percent of all U.S. deaths are now due to medical error. - Click to Tweet - (http://ctt.ec/6UDuI)
- Third highest cause of death in the U.S. is medical error.- Click to Tweet - (http://ctt.ec/v61RG)
- Medical errors are an under-recognized cause of death. - Click to Tweet - (http://ctt.ec/AD8cS)

Analyzing medical death rate data over an eight-year period, Johns Hopkins patient safety experts have calculated that more than 250,000 deaths per year are due to medical error in the U.S. Their figure, published May 3 in *The BMJ*, surpasses the U.S. Centers for Disease Control and Prevention's (CDC's) third leading cause of death — respiratory disease - (http://www.hopkinsmedicine.org/healthlibrary/conditions/adult/respiratory_disorders/smoking_and_respiratory_diseases_85,P01331), which kills close to 150,000 people per year.

The Johns Hopkins team says the CDC's way of collecting national health statistics fails to classify medical errors separately on the death certificate. The researchers are advocating for updated criteria for classifying deaths on death certificates.

"Incidence rates for deaths directly attributable to medical care gone awry haven't been recognized in any standardized method for collecting national statistics," says Martin Makary, M.D., M.P.H. - (https://www.hopkinsmedicine.org/profiles/results/directory/profile/0018306/martin-makary), professor of surgery at the Johns Hopkins University School of Medicine and an authority on health reform. "The medical coding system was designed to maximize billing for physician services, not to collect national health statistics, as it is currently being used."

In 1949, Makary says, the U.S. adopted an international form that used International Classification of Diseases (ICD) billing codes to tally causes of death.

"At that time, it was under-recognized that diagnostic errors, medical mistakes and the absence of safety nets could result in someone's death, and because of that, medical errors were unintentionally excluded from national health statistics," says Makary.

The researchers say that since that time, national mortality statistics have been tabulated using billing codes, which don't have a built-in way to recognize incidence rates of mortality due to medical care gone wrong.

In their study, the researchers examined four separate studies that analyzed medical death rate data from 2000 to 2008, including one by the U.S. Department of Health and Human Services' Office of the Inspector General and the Agency for Healthcare Research and Quality. Then, using hospital admission rates from 2013, they extrapolated that based on a total of 35,416,020 hospitalizations, 251,454 deaths stemmed from a medical error, which the researchers say now translates to 9.5 percent of all deaths each year in the U.S.

According to the CDC - (http://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm), in 2013, 611,105 people died of heart disease - (http://www.hopkinsmedicine.org/healthlibrary/conditions/adult/cardiovascular_diseases/coronary_heart_disease_85,P00207/), 584,881 died of cancer and 149,205 died of chronic respiratory disease — the top three causes of death in the U.S. The newly calculated figure for medical errors puts this cause of death behind cancer but ahead of respiratory disease.

"Top-ranked causes of death as reported by the CDC inform our country's research funding and public health priorities," says Makary. "Right now, cancer and heart disease get a ton of attention, but since medical errors don't appear on the list, the problem doesn't get the funding and attention it deserves."

The researchers caution that most of medical errors aren't due to inherently bad doctors, and that reporting these errors shouldn't be addressed by punishment or legal action. Rather, they say, most errors represent systemic problems, including poorly coordinated care, fragmented insurance networks, the absence or underuse of safety nets, and other protocols, in addition to unwarranted variation in physician practice patterns that lack accountability.

"Unwarranted variation is endemic in health care. Developing consensus protocols that streamline the delivery of medicine and reduce variability can improve quality and lower costs in health care. More research on preventing medical errors from occurring is needed to address the problem," says Makary.

Michael Daniel of Johns Hopkins is a co-author on the study.

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EXHIBIT 394

Vaccine Coverage Levels – United States, 1962-2016

Year	DTP 3+	DTP4+	Polio 3+	MMR*	Hib3+	Var	PCV3+	HepB3+	Rota	Combined 4-3-1	Combined 4-3-1-3
1962	67.3										
1963	71.4										
1964	74.6										
1965	72.7										
1966	74.0										
1967	77.9			60.0							
1968	76.8			61.5							
1969	77.4			61.4							
1970	76.4			58.4							
1971	77.8			62.2							
1972	74.1			62.8							
1973	71.7		59.5	61.0							
1974	72.4		60.0	63.4							
1975	73.2		63.6	65.5							
1976	72.7		61.3	66.3							
1977	69.6		62.6	65.0							
1978	66.6		59.5	63.6							
1979	64.4		59.7	66.5							
1980	66.0		58.9	66.6							
1981	68.1		59.2	66.8							
1982	67.1		57.0	67.6							
1983	65.4		56.9	66.3							
1984	65.0		53.2	65.8							
1985	63.6		53.6	61.2							
1986†											
1987†											
1988†											
1989†											
1990†											
1991	68.8		53.2	82.0							
1992	83.0	59.0	72.4	82.5	28.2			8.0		68.7	55.3
1993	88.2	72.1	78.9	84.1	55.0			16.3		67.1	
1994	93.0	77.7	83.0	89.0	86.0			37.0		75.0	
1995	94.7	78.5	87.9	87.6	91.7			68.0		76.2	74.2
1996	95.0	81.1	91.1	90.7	91.7	16.0		81.8		78.4	76.5
1997	95.5	81.5	90.8	90.5	92.7	25.9		83.7		77.9	76.2
1998	95.6	83.9	90.8	92.0	93.4	43.2		87.0		80.6	79.2
1999	95.9	83.3	89.6	91.5	93.5	57.5		88.1		79.9	78.4
2000	94.1	81.7	89.5	90.5	93.4	67.8		90.3		77.6	76.2
2001	94.3	82.1	89.4	91.4	93.0	76.3		88.9		78.6	77.2
2002	94.9	81.6	90.2	91.6	93.1	80.6	40.8	88.9		78.5	77.5
2003	96.0	84.8	91.6	93.0	93.9	84.8	68.1	92.4		82.2	81.3
2004	95.9	85.5	91.6	93.0	93.5	87.5	73.2	92.4		83.5	82.5
2005	96.1	85.7	91.7	91.5	93.9	87.9	82.8	92.9		83.1	82.4
2006	95.8	85.2	92.9	92.4	93.4	89.3	87.0	93.4		83.2	82.3
2007	95.5	84.5	92.6	92.3	92.6	90.0	90.0	92.7		82.8	81.1
2008	96.2	84.6	93.6	92.1	90.9	90.7	92.8	93.5		82.5	79.6
2009	94.0	83.9	92.8	90.0	92.1	89.6	92.6	92.4	43.9	81.5	50.6
2010	95.0	84.4	93.3	91.5	90.4	90.4	92.6	91.8	59.2	82.0	78.8
2011	95.5	84.6	93.9	91.6	94.0	90.8	93.6	91.1	67.3	82.6	81.9
2012	94.3	82.5	92.8	90.8	93.0	90.2	92.3	89.7	68.6	80.5	76.0
2013	94.1	83.1	92.7	91.9	92.8	91.2	92.4	90.8	72.6	81.5	77.1
2014	94.7	84.2	93.3	91.5	92.6	91.0	92.6	91.6	71.7	82.6	77.7
2015	95.0	84.6	93.7	91.9	93.2	91.8	93.3	92.6	73.2	83.2	77.7
2016	93.7	83.4	91.1	91.1	91.6	90.6	91.8	90.5	74.1	81.9	76.8

*Previously reported as measles-containing vaccine (MCV)
†No national coverage data were collected from 1986 through 1990.

Combined 4-3-1: Four or more doses of DTP/DTaP/DT, three or more doses of poliovirus vaccine, and one or more doses of any measles-containing vaccine.

Combined 4-3-1-3: Four or more doses of DTP/DTaP/DT, three or more doses of poliovirus vaccine, one or more doses of any measles-containing vaccine, and three or more doses of *Haemophilus influenzae* type b vaccine.

Data prior to 1993 were collected by the National Health Interview Survey and represent 2-year-old children. Data from 1993 forward are from the National Immunization Survey and represent 19-35 month-old children. Different methods were used for the two surveys.

Data are available for vaccines and combinations of vaccines not reflected on this table. For more information about annual coverage figures from 1995 to the present, see https://www.cdc.gov/vaccines/imz-managers/coverage/childvaxview/data-reports/index.html .

EXHIBIT 395

MMWR Weekly
August 8, 2003 / 52(31);728-732

National, State, and Urban Area Vaccination Levels Among Children Aged 19--35 Months --- United States, 2002

Each annual birth cohort in the United States comprises approximately four million infants. Maintaining the gains in childhood vaccination coverage achieved during the 1990s among these children poses an ongoing challenge for public health. The National Immunization Survey (NIS) provides annual estimates of vaccination coverage among children aged 19--35 months for each of the 50 states and 28 selected urban areas*. This report presents NIS findings for 2002†, which indicate a marked nationwide increase in coverage with ≥1 dose of varicella vaccine (VAR), substantial uptake for ≥3 doses of pneumococcal conjugate vaccine (PCV), generally steady coverage levels for other vaccines nationwide, and continued wide variability in coverage among the states and selected urban areas.

To collect vaccination data for all age-eligible children, NIS uses a quarterly random-digit--dialing sample of telephone numbers for each of the 78 survey areas. NIS methodology, including how the responses are weighted to represent the population of children aged 19--35 months, has been described previously (*1*,*2*). During 2002, health-care provider vaccination records were obtained for 21,317 children. The overall response rate for eligible households in 2002 was 62.3%.

National vaccination coverage with ≥1 dose of VAR increased from 76.3% (95% confidence interval [CI] = ±0.8%) in 2001 to 80.6% (95% CI = ±0.9%) in 2002. Coverage for ≥3 doses of PCV, reported for the first time, was 40.9% (95% CI = ±1.1%). For all other vaccines, coverage levels remained steady during 2001--2002. For all combined vaccine series reported previously, coverage remained steady (Table 1). In 2002, coverage was reported for the 4:3:1:3:3:1§ series, which includes ≥1 dose of VAR. Coverage in 2002 for the 4:3:1:3:3:1 series was 65.5% (95% CI = ±1.1%), compared with 2000 and 2001, when coverage for this series was 54.1% (95% CI = ±1.0%) and 61.3% (95% CI = ±1.0%), respectively (Table 1).

In 2002, substantial differences remained in estimated vaccination coverage among the states. The estimated coverage with the 4:3:1:3:3¶ series ranged from 86.2% in Massachusetts to 62.7% in Colorado (Table 2). Variability among the 28 selected urban areas was slightly less than that among the states. Among

the 28 selected urban areas, the highest estimated coverage for the 4:3:1:3:3 series ranged from 81.1% in Santa Clara County, California, to 57.5% in Newark, New Jersey (Table 2).

Reported by: *L Barker, PhD, N Darling, MPH, Data Management Div; M McCauley, MTSC, Office of the Director; J Santoli, MD, Immunization Svcs Div, National Immunization Program, CDC.*

Editorial Note:

The findings in the report indicate that among U.S. children aged 19--35 months, coverage with the recommended vaccines in 2002 remained near all-time highs. Changes in national level coverage from 2001 to 2002 with all vaccines other than VAR and PCV were so small that they are unlikely to have a major public health impact. Although coverage with recommended vaccines for each new birth cohort remains high, vigilance is needed to maintain these high levels. Eliminating the coverage disparity between states and urban areas with the highest and lowest coverage remains a priority. If vaccine-preventable disease is introduced in an area with low coverage, groups of susceptible children might serve as a reservoir to transmit disease.

Because coverage with ≥1 dose of VAR attained a level approximately equal to that of ≥4 doses of DTaP, coverage for the 4:3:1:3:3:1 series, which includes VAR, was assessed and presented for the first time in this report. From 2000 to 2002, steady increases were observed. The 2002 NIS cohort was the first entire NIS birth cohort to be eligible for PCV. Coverage with ≥3 doses of PCV (40.9%) was similar to coverage for VAR in 1998 (43.2%), the first year for which the entire NIS birth cohort was eligible for that vaccine. Uptake for ≥3 doses of PCV showed steady quarterly increases (Q1 = 24.5%; Q2 = 35.3%; Q3 = 48.8%; Q4 = 56.3%), with a similar trend for ≥4 doses.

The findings in this report are subject to at least three limitations. First, NIS is a telephone survey; although statistical weights adjust for nonresponse and households without telephones, some bias might remain. Second, although NIS relies on provider-verified vaccination histories, incomplete records and reporting could result in underestimates of coverage. The estimation procedure assumes that coverage among children whose providers do not respond is similar to that among children whose providers respond. Finally, although national level estimates are precise, estimates for states and urban areas should be interpreted with caution (*3*); CIs are wider for state and selected urban areas compared with national estimates.

During the time that children in the 2002 cohort were to be vaccinated, vaccines in short supply included DTaP; measles, mumps, and rubella (MMR); VAR; and PCV (*4*--*7*). When DTaP was in short supply, approximately 86% of the NIS cohort needed ≥1 dose of the vaccine to stay on schedule. For MMR, VAR, and PCV, the percentages were approximately 6%, 21%, and 37%, respectively. NIS has sufficient power to detect a moderate (e.g., 15%) decrease in coverage even among the 6% of children due to receive a dose of MMR during the period it was in short supply; no effect on coverage was noted for any vaccine or series. These shortages affected children, their parents, and health-care providers; however, many aspects of vaccine delivery are not reflected by coverage attained among children aged 19--35 months. For example, if vaccine was unavailable at a health-care provider visit, another visit could have been made at a later time when vaccine was obtained. Such affected children, although lacking optimal protection for some period, still could show up as fully vaccinated through NIS. The impact of the shortages also might have been minimized if efforts by health-care providers, such as recalling children who missed doses and administering catch-up doses, had taken place. Further analysis of the 2002 data are ongoing to assess these potential impacts of the shortages, including changes in the percentage of children who received vaccines at

recommended ages or the number of health-care provider visits required for children to be vaccinated fully. Health-care providers serving the cohort of children surveyed in 2002 also might have mitigated the effects of the shortages with vaccines already on hand that had been distributed during 1999--2001. Because many children affected by the shortages will be members of the 2003 NIS birth cohort, potential impacts on coverage and timeliness should be assessed in next year's data.

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* Jefferson County, Alabama; Maricopa County, Arizona; Los Angeles, San Diego, and Santa Clara counties, California; District of Columbia; Miami-Dade and Duval counties, Florida; Fulton/DeKalb counties, Georgia; Chicago, Illinois; Marion County, Indiana; Orleans Parish, Louisiana; Baltimore, Maryland; Boston, Massachusetts; Detroit, Michigan; Newark, New Jersey; New York, New York; Cuyahoga and Franklin counties, Ohio; Philadelphia County, Pennsylvania; Davidson and Shelby counties, Tennessee; Bexar, Dallas, and El Paso counties, and Houston, Texas; King County, Washington; and Milwaukee County, Wisconsin.

† For the January--December 2002 reporting period, NIS included children born during February 1999--June 2001.

§ Comprises ≥4 doses of diphtheria and tetanus toxoids and pertussis vaccine, diphtheria and tetanus toxoids, and diphtheria and tetanus toxoids and acellular pertussis vaccine (DTP/DT/DTaP); ≥3 doses of poliovirus vaccine; ≥1 dose of measles-containing vaccine (MCV); ≥3 doses of *Haemophilus influenzae* type b vaccine (Hib); ≥3 doses of hepatitis B vaccine (hep B); and ≥1 dose of VAR vaccine.

¶ Comprises ≥4 doses of DTP vaccine, ≥3 doses of poliovirus vaccine, ≥1 dose of MCV, ≥3 doses of Hib vaccine, and ≥3 doses of hepB vaccine.

Table 1

TABLE 1. Vaccination coverage levels among children aged 19–35 months, by selected vaccines — National Immunization Survey, United States, 1998–2002

Vaccine/Dose	1998* %	1998* (95% CI††)	1999† %	1999† (95% CI)	2000§ %	2000§ (95% CI)	2001¶ %	2001¶ (95% CI)	2002** %	2002** (95% CI)
DTP/DT/DTaP§§										
≥3 doses	95.6	(±0.5)	95.9	(±0.4)	94.1	(±0.5)	94.3	(±0.5)	94.9	(±0.6)
≥4 doses	83.9	(±0.8)	83.8	(±0.8)	81.7	(±0.8)	82.1	(±0.8)	81.6	(±0.9)
Poliovirus										
≥3 doses	90.8	(±0.7)	89.6	(±0.6)	89.5	(±0.6)	89.4	(±0.7)	90.2	(±0.7)
Hib¶¶										
≥3 doses	93.4	(±0.6)	93.5	(±0.5)	93.4	(±0.5)	93.0	(±0.6)	93.1	(±0.6)
MMR***										
≥1 dose	92.0	(±0.6)	91.5	(±0.6)	90.5	(±0.6)	91.4	(±0.6)	91.6	(±0.7)
Hepatitis B										
≥3 doses	87.0	(±0.7)	88.1	(±0.7)	90.3	(±0.6)	88.9	(±0.7)	89.9	(±0.7)
Varicella										
≥1 dose	43.2	(±1.0)	57.5	(±1.0)	67.8	(±0.9)	76.3	(±0.8)	80.6	(±0.9)
PCV†††										
≥3 doses	—		—		—		—		40.9	(±1.1)
Combined series										
4:3:1§§§	80.6	(±0.9)	79.9	(±0.8)	77.6	(±0.9)	78.6	(±0.9)	78.5	(±1.0)
4:3:1:3¶¶¶	79.2	(±0.9)	78.4	(±0.9)	76.2	(±0.9)	77.2	(±0.9)	77.5	(±1.0)
4:3:1:3:3****	—		73.2	(±0.9)	72.9	(±0.9)	73.7	(±0.9)	74.8	(±1.0)
4:3:1:3:3:1††††	—		—		54.1	(±1.0)	61.3	(±1.0)	65.5	(±1.1)

* Born during February 1995–June 1997.
† Born during February 1996–June 1998.
§ Born during February 1997–June 1999.
¶ Born during February 1998–June 2000.
** Born during February 1999–June 2001.
†† Confidence interval.
§§ Diphtheria and tetanus toxoids and pertussis vaccine, diphtheria and tetanus toxoids, and diphtheria and tetanus toxoids and acellular pertussis vaccine.
¶¶ *Haemophilus influenzae* type b.
*** Measles, mumps, and rubella vaccine.
††† Pneumococcal conjugate vaccine.
§§§ Comprises ≥4 doses of DTP/DT/DTaP, ≥3 doses of poliovirus vaccine, and ≥1 dose of measles-containing vaccine.
¶¶¶ 4:3:1 plus ≥3 doses of Hib vaccine.
**** 4:3:1:3 plus ≥3 doses of hepatitis B vaccine.
†††† 4:3:1:3:3 plus ≥1 dose of varicella vaccine.

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Table 2

TABLE 2. Estimated vaccination coverage levels with 4:3:1*, 4:3:1:3†, 4:3:1:3:3§, and 4:3:1:3:3:1¶ series among children aged 19–35 months, by states and selected urban areas — National Immunization Survey, United States, 2002

State/Urban area	4:3:1		4:3:1:3		4:3:1:3:3		4:3:1:3:3:1	
	%	(95% CI**)	%	(95% CI)	%	(95% CI)	%	(95% CI)
Alabama	80.8	(±5.1)	79.5	(±5.1)	76.8	(±5.3)	73.3	(±5.5)
Jefferson County	81.7	(±5.4)	81.7	(±5.4)	77.8	(±5.9)	74.1	(±6.2)
Rest of state	80.6	(±5.9)	79.2	(±6.0)	76.6	(±6.1)	73.1	(±6.4)
Alaska	78.3	(±5.6)	78.3	(±5.6)	75.3	(±5.9)	56.2	(±6.7)
Arizona	70.0	(±4.7)	69.5	(±4.7)	67.9	(±4.7)	59.0	(±4.9)
Maricopa County	73.7	(±6.3)	73.1	(±6.3)	71.8	(±6.4)	62.2	(±6.7)
Rest of state	63.5	(±6.8)	63.3	(±6.8)	61.2	(±6.7)	53.5	(±6.8)
Arkansas	74.6	(±5.9)	74.4	(±5.9)	71.0	(±6.1)	68.3	(±6.4)
California	77.5	(±3.7)	75.8	(±3.8)	73.2	(±3.8)	67.1	(±4.0)
Los Angeles County	79.6	(±5.6)	77.1	(±5.8)	76.0	(±5.9)	72.3	(±6.1)
San Diego County	79.0	(±5.7)	77.7	(±5.8)	74.1	(±6.1)	70.7	(±6.3)
Santa Clara County	85.0	(±4.4)	83.7	(±4.5)	81.1	(±4.8)	75.2	(±5.3)
Rest of state	75.6	(±5.7)	74.0	(±5.8)	70.9	(±5.9)	63.1	(±6.2)
Colorado	64.7	(±6.6)	64.3	(±6.6)	62.7	(±6.6)	56.1	(±6.8)
Connecticut	86.1	(±4.8)	85.7	(±4.9)	81.9	(±5.2)	72.8	(±5.9)
Delaware	84.8	(±4.6)	81.1	(±5.3)	78.7	(±5.5)	69.7	(±5.9)
District of Columbia	73.8	(±7.4)	72.2	(±7.4)	69.7	(±7.5)	68.3	(±7.5)
Florida	78.0	(±4.4)	77.2	(±4.4)	74.5	(±4.7)	66.4	(±5.1)
Miami-Dade County	75.4	(±6.3)	73.3	(±6.4)	70.9	(±6.5)	60.2	(±7.0)
Duval County	78.0	(±6.9)	77.3	(±6.9)	76.1	(±7.0)	70.3	(±7.1)
Rest of state	78.6	(±5.5)	78.0	(±5.5)	75.1	(±5.8)	67.3	(±6.4)
Georgia	83.4	(±3.9)	82.0	(±4.1)	80.4	(±4.2)	76.5	(±4.5)
Fulton/DeKalb counties	79.4	(±5.6)	79.1	(±5.6)	77.5	(±5.7)	74.6	(±5.9)
Rest of state	84.4	(±4.7)	82.6	(±4.9)	81.0	(±5.0)	76.9	(±5.4)
Hawaii	81.3	(±5.4)	80.9	(±5.4)	78.7	(±5.5)	69.1	(±6.1)
Idaho	73.9	(±5.7)	73.3	(±5.8)	69.4	(±5.9)	52.6	(±6.3)
Illinois	80.4	(±4.2)	79.6	(±4.3)	78.6	(±4.3)	58.1	(±5.3)
Chicago	72.3	(±7.4)	71.5	(±7.4)	69.1	(±7.5)	58.3	(±7.9)
Rest of state	83.5	(±5.1)	82.6	(±5.1)	82.1	(±5.2)	58.1	(±6.6)
Indiana	79.2	(±4.5)	77.9	(±4.6)	76.0	(±5.0)	59.4	(±5.8)
Marion County	75.6	(±6.5)	75.3	(±6.5)	74.0	(±6.5)	62.2	(±7.0)
Rest of state	79.9	(±5.2)	78.4	(±5.4)	76.4	(±5.8)	58.9	(±6.8)
Iowa	80.7	(±5.4)	79.7	(±5.4)	78.7	(±5.5)	58.2	(±6.5)
Kansas	74.0	(±6.6)	72.9	(±6.6)	66.8	(±6.9)	55.1	(±6.9)
Kentucky	74.4	(±6.3)	74.4	(±6.3)	72.3	(±6.4)	63.6	(±6.8)
Louisiana	69.8	(±5.5)	69.3	(±5.5)	66.8	(±5.6)	61.9	(±5.8)
Orleans Parish	65.0	(±8.0)	63.4	(±8.1)	60.5	(±8.3)	53.3	(±8.6)
Rest of state	70.4	(±6.2)	70.0	(±6.2)	67.6	(±6.3)	63.0	(±6.4)
Maine	83.7	(±4.9)	82.8	(±4.9)	80.7	(±5.1)	62.1	(±6.5)
Maryland	81.8	(±5.5)	80.8	(±5.6)	78.7	(±5.6)	70.7	(±6.4)
Baltimore	76.2	(±6.3)	74.6	(±6.3)	70.8	(±6.7)	69.1	(±6.8)
Rest of state	82.7	(±6.4)	81.9	(±6.4)	80.1	(±6.5)	71.0	(±7.3)
Massachusetts	89.5	(±3.4)	89.2	(±3.4)	86.2	(±3.8)	78.0	(±4.6)
Boston	82.5	(±5.3)	79.9	(±5.6)	76.6	(±6.3)	70.7	(±6.5)
Rest of state	90.3	(±3.7)	90.3	(±3.7)	87.4	(±4.1)	78.8	(±5.0)
Michigan	84.3	(±4.1)	83.8	(±4.2)	81.6	(±4.4)	71.7	(±5.6)
Detroit	66.7	(±6.8)	65.9	(±6.8)	64.5	(±6.8)	59.5	(±6.9)
Rest of state	86.6	(±4.6)	86.1	(±4.6)	83.9	(±4.9)	73.3	(±6.3)
Minnesota	82.2	(±5.6)	78.9	(±6.5)	76.8	(±6.5)	61.5	(±6.9)
Mississippi	77.8	(±6.2)	77.8	(±6.2)	75.7	(±6.5)	63.9	(±7.3)
Missouri	77.7	(±6.3)	77.3	(±6.4)	73.0	(±6.5)	60.1	(±7.0)
Montana	71.5	(±6.6)	70.9	(±6.7)	66.6	(±6.8)	49.4	(±7.2)
Nebraska	80.6	(±5.4)	79.2	(±5.5)	78.2	(±5.6)	64.3	(±6.3)
Nevada	78.4	(±5.9)	77.8	(±6.0)	76.4	(±6.1)	65.3	(±6.5)
New Hampshire	88.1	(±4.4)	87.3	(±4.5)	83.5	(±5.0)	66.2	(±6.5)

* Comprises ≥4 doses of diphtheria and tetanus toxoids and pertussis vaccine, diphtheria and tetanus toxoids, and diphtheria and tetanus toxoids and acellular pertussis vaccine; ≥3 doses of poliovirus vaccine; and ≥1 dose of measles-containing vaccine.
† 4:3:1 plus ≥3 doses of *Haemophilus influenzae* type b vaccine.
§ 4:3:1:3 plus ≥3 doses of hepatitis B vaccine.
¶ 4:3:1:3:3 plus ≥1 dose of varicella vaccine.
** Confidence interval.

TABLE 2. (*Continued*) Estimated vaccination coverage levels with 4:3:1*, 4:3:1:3†, 4:3:1:3:3§, and 4:3:1:3:3:1¶ series among children aged 19–35 months, by states and selected urban areas — National Immunization Survey, United States, 2002

State/Urban area	4:3:1 %	4:3:1 (95% CI**)	4:3:1:3 %	4:3:1:3 (95% CI)	4:3:1:3:3 %	4:3:1:3:3 (95% CI)	4:3:1:3:3:1 %	4:3:1:3:3:1 (95% CI)
New Jersey	81.9	(±4.9)	80.4	(±5.0)	76.1	(±5.4)	65.5	(±6.0)
Newark	61.5	(±8.2)	59.9	(±8.2)	57.5	(±8.1)	50.4	(±7.9)
Rest of state	82.9	(±5.1)	81.3	(±5.2)	77.0	(±5.7)	66.2	(±6.3)
New Mexico	68.1	(±6.6)	67.4	(±6.6)	64.6	(±6.7)	59.1	(±7.0)
New York	81.8	(±4.0)	81.3	(±4.0)	77.5	(±4.3)	67.3	(±4.8)
New York City	81.8	(±5.8)	81.0	(±5.9)	78.1	(±6.2)	71.0	(±6.7)
Rest of state	81.8	(±5.5)	81.6	(±5.5)	77.0	(±6.0)	64.0	(±6.8)
North Carolina	86.9	(±4.9)	86.5	(±4.9)	82.4	(±5.5)	69.7	(±6.8)
North Dakota	78.8	(±6.7)	78.8	(±6.7)	77.7	(±6.7)	56.3	(±6.9)
Ohio	77.9	(±4.4)	77.1	(±4.4)	75.0	(±4.5)	63.5	(±4.9)
Cuyahoga County	74.6	(±7.7)	74.2	(±7.8)	72.1	(±7.8)	65.0	(±8.0)
Franklin County	84.5	(±5.2)	83.7	(±5.2)	81.0	(±5.6)	69.4	(±6.8)
Rest of state	77.5	(±5.5)	76.6	(±5.5)	74.6	(±5.7)	62.4	(±6.1)
Oklahoma	69.6	(±7.1)	66.7	(±7.4)	65.3	(±7.4)	60.3	(±7.4)
Oregon	74.8	(±5.6)	74.5	(±5.6)	70.0	(±5.9)	60.3	(±6.1)
Pennsylvania	78.7	(±5.2)	77.1	(±5.3)	74.7	(±5.5)	67.6	(±5.8)
Philadelphia County	75.0	(±6.0)	73.5	(±6.0)	72.0	(±6.1)	68.2	(±6.3)
Rest of state	79.3	(±6.0)	77.7	(±6.2)	75.2	(±6.4)	67.5	(±6.7)
Rhode Island	90.1	(±4.1)	85.8	(±5.5)	84.5	(±5.6)	80.7	(±5.9)
South Carolina	80.5	(±6.4)	80.2	(±6.4)	78.8	(±6.5)	73.8	(±6.7)
South Dakota	82.0	(±6.3)	81.2	(±6.3)	79.9	(±6.4)	62.0	(±7.0)
Tennessee	80.5	(±3.9)	79.7	(±4.0)	78.2	(±4.1)	67.3	(±4.8)
Davidson County	81.3	(±5.8)	79.8	(±6.1)	79.3	(±6.2)	66.7	(±7.3)
Shelby County	73.4	(±6.7)	72.6	(±6.7)	72.5	(±6.7)	60.6	(±7.2)
Rest of state	82.3	(±5.2)	81.5	(±5.3)	79.6	(±5.4)	69.2	(±6.5)
Texas	71.3	(±5.0)	70.9	(±5.0)	67.9	(±5.1)	65.0	(±5.1)
Bexar County	76.4	(±5.8)	75.9	(±5.8)	73.9	(±5.9)	71.8	(±6.1)
Houston	64.2	(±8.0)	63.9	(±8.1)	61.4	(±8.0)	55.6	(±8.0)
Dallas County	77.3	(±5.1)	75.9	(±5.2)	71.5	(±5.5)	68.0	(±5.8)
El Paso County	78.6	(±5.9)	77.1	(±6.0)	67.4	(±7.1)	60.6	(±7.3)
Rest of state	70.6	(±7.4)	70.4	(±7.4)	67.8	(±7.5)	65.8	(±7.5)
Utah	79.9	(±5.6)	79.1	(±5.6)	75.7	(±5.9)	61.4	(±6.5)
Vermont	87.7	(±3.9)	87.0	(±4.0)	80.9	(±4.7)	57.7	(±6.3)
Virginia	77.7	(±5.8)	76.6	(±5.9)	72.0	(±6.2)	64.8	(±6.5)
Washington	74.7	(±4.7)	73.1	(±4.9)	69.2	(±5.0)	51.9	(±5.1)
King County	78.3	(±5.3)	76.9	(±5.4)	73.1	(±5.6)	56.3	(±6.3)
Rest of state	73.3	(±6.2)	71.7	(±6.4)	67.7	(±6.5)	50.2	(±6.6)
West Virginia	79.0	(±6.1)	78.5	(±6.2)	76.9	(±6.3)	65.8	(±6.8)
Wisconsin	83.4	(±4.2)	81.8	(±4.3)	80.3	(±4.3)	67.5	(±5.0)
Milwaukee County	73.6	(±7.3)	69.8	(±7.6)	67.8	(±7.7)	59.9	(±7.7)
Rest of state	86.2	(±4.9)	85.2	(±5.0)	83.9	(±5.1)	69.6	(±6.0)
Wyoming	76.5	(±6.1)	76.5	(±6.1)	73.3	(±6.4)	54.1	(±6.8)
Total	**78.5**	**(±1.0)**	**77.5**	**(±1.0)**	**74.8**	**(±1.0)**	**65.5**	**(±1.1)**

* Comprises ≥4 doses of diphtheria and tetanus toxoids and pertussis vaccine, diphtheria and tetanus toxoids, and diphtheria and tetanus toxoids and acellular pertussis vaccine; ≥3 doses of poliovirus vaccine; and ≥1 dose of measles-containing vaccine.
† 4:3:1 plus ≥3 doses of *Haemophilus influenzae* type b vaccine.
§ 4:3:1:3 plus ≥3 doses of hepatitis B vaccine.
¶ 4:3:1:3:3 plus ≥1 dose of varicella vaccine.
** Confidence interval.

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