TO: DR. GORDON DOUGLAS RY 33-76

FR: Maurice R. Hilleman WP 26-200B

RE: VACCINE TASK FORCE ASSIGNMENT
THIMEROSAL (MERTHIOLATE) PRESERVATIVE -
PROBLEMS, ANALYSIS, SUGGESTIONS FOR RESOLUTION

1. PROBLEM.

The regulatory control agencies in some countries, particularly Scandinavia (especially Sweden), but also U.K., Japan, and Switzerland, have expressed concern for thimerosal, a mercurial preservative, in vaccines.

Some countries require absence of thimerosal from single-dose vials and prefer to buy vaccines in the single-dose package. This trend will probably spread. Thimerosal is allowed where multidose vials are the only alternative.

Sweden is requiring thimerosal-free single-dose packaging of all products, as soon as can be reasonably achieved. The deadline for DT is January, 1992. Competitor HibTITER (free of thimerosal) will be chosen for *Haemophilus influenzae* vaccination until alternative thimerosal-free packaged vaccines are available.

The U.S. Food & Drug Administration (CBER) does not have this concern for thimerosal but will permit exclusion from single-dose vials if requested and qualified. Misuse of single-dose vials by multiple puncture to achieve more does (*e.g.*, 2.5 µg quantities for infants from 10 µg adult product) is the user's responsibility and ends with the requirement that the labelling clearly states that the vial contains a single dose and that the vial is not to be reentered.

The key issue is whether thimerosal, in the amount given with the vaccine, does or does not constitute a safety hazard. However, perception of hazard may be equally important.

The basis for concern, assessment of hazard, and suggested resolution are given below.

2. COMPOSITION OF THIMEROSAL (Merthiolate®).

The 1989 Merck Index gives the following description:

9344. Thimerosal. [illegible] sodium ethylmercurithiosalicylate; [illegible]; Merthiolate; Merzonin; Mertorgan; [illegible]. $C_9H_9HgNaO_2S$; mol wt 404.8[illegible]. C 26.70%, H 2.24%, Hg 49.5[illegible]%, Na 5.[illegible]%, O 7.90%, S 7.92%. Prepd by reacting ethylmercuric chloride (or ethylmercuric hydroxide) with thiosalicylic acid: Kharasch, U.S. pat. 1,672,615 (1928); [illegible], Nature 158, 472 (1946); Swirska et al., Przemysl Chem. 39, 171 (1960), C.A. 55, 23076 (1961). Toxicity: Mason et al. Clin. Toxicol. 4, 185 (1971).

Stabilization of solns with EDTA: Davisson, U.S. pat. 2,846,364 (1958 to Lilly). pH of 1% aq soln: 6.7. LD_{50} s.c. in rats: 98 mg/kg (Mason).

USE: Pharmaceutic aid (preservative).

THERAP CAT: Anti-infective.

THERAP CAT (VET): Antibacterial, antifungal (topical).

Cream-colored, crystalline powder. Stable in air, but not in sunlight. One gram dissolves in about 1 ml water, in about 8 ml alcohol. Practically insol in ether and benzene.

From the pharmaco-toxicologic standpoint, it is important to note that thimerosal is the sodium salt of a phenolic acid with a thio-ethyl-mercurial side chain. Almost half, 49.55%, of the weight of thimerosal is mercury.

Thimerosal is used as an antimicrobial preservative in vaccines, usually at a concentration of 1:10,000 by weight, though less often at 1:20,000.

3. WHY THE CONCERN? THIMEROSAL HAS BEEN USED FOR DECADES.

The focal point for present concern is in Scandinavia, though the USSR was probably the first. The immediate Merck concern is to be able to qualify for sale of single-dose products in Sweden and in Norway and Denmark.

The State Bacteriological Laboratory in Stockholm, which is highly competent, does not feel a high-level urgency to solve the thimerosal problem since the amount of mercury from vaccine sources is considered to be inconsequential compared with the intake from air, fish, dental amalgams, etc.

However, there is an active response to a public perception and, hence, concern for thimerosal in vaccines. The public awareness has been raised by the sequential wave of experiences in Sweden including mercury exposure from additives, fish, contaminated air, bird deaths from eating mercury-treated seed grains, dental amalgam leakage, mercury allergy, etc.

The target for the Swedish licensing authority is to use single-dose vials of vaccine and to make available products that are not preserved with thimerosal. Where thimerosal-free vaccine is not available, *e.g.*, hepatitis B vaccine, then thimerosal-containing product will be allowed until a thimerosal-free source does become available. In some instances, public immunization programs may be endangered by public refusal to accept vaccines with thimerosal.

The State Bacteriological Laboratory does have a program to find and qualify a suitable substitute for thimerosal, but there has been no substantial progress to date.

4. CLINICAL CONCERNS

a. Allergies. The published literature records a number of instances of allergies in patients sensitized with organic mercurials, including thimerosal. Cross-sensitization of people between different organic mercurials is noted. A common means for sensitization of people is by use of contact lens fluids preserved with thimerosal. Reported reactions to thimerosal-preserved vaccines includs eczema, generalized exanthems and urticaria.

b. Amalgam restorations. Mercury released from dental amalgams has been held responsible (probably spuriously in most instances) for a variety of maladies including multiple sclerosis, chronic fatigue syndrome, allergic sensitization, autoimmune disease, etc.

Markert has recently undertaken studies to determine whether the mercury released from dental amalgams influences the immune system as measured by change in lymphocyte subset counts. Comparison was made between subjects with or without exposed amalgam. There were no significant differences in the two groups as relates to T3, T4, T8, T11, B1 or Leu 7 cells. Markert estimated that the average daily release of mercury from 8 occlusal surfaces to be 1.2 μg.

It may be of interest that Andersson *et al.* in the State Bacteriological Laboratories in Stockholm have recently reported *in vitro* activation by phenyl mercury of T cells from persons with amalgam who are allergic to mercury.

5. RELATIVE TOXICITY OF MERCURIALS.

From the toxicologic standpoint, mercury and compounds of mercury are divided into 3 groups:

a. Methyl and ethyl mercury salts (this is the most toxic form).

b. Mercury vapor (intermediate).

c. Inorganic mercury salts, and phenyl and methoxyethyl mercury salts. (These may differ within the group but are considered least toxic.)

An International Committee (1969) ranked the 3 above classes according to allowable 8-hour exposure to an amount of mercury per cubic meter of air.

The values were:

Methyl and ethyl mercury salts -	.01 mg/cubic meter
Mercury vapor	.05 mg/cubic meter
Inorganic salts, phenyl & methoxy	0.1 mg/cubic meter

These are air exposure values but probably reflect the relative hazard of the different forms of mercury.

It is important that group 3 is least toxic and is perhaps 1/10 as toxic as the methyl and ethyl mercurial salts. Most important, thimerosal is a phenyl mercurial.

6. TOXICOLOGIC ASSESSMENT OF THE HAZARD OF THIMEROSAL IN THE AMOUNT USED.

a. Literature review has revealed no toxic-pharmacologic study of thimerosal except for a paper by Mason *et al.* who did comparative toxicologic studies of 7 different chemicals used in preparing vaccines. The single-dose LD_{50} in mg/kg in weanling rats was 119 for Benzethonium chloride and 98 for thimerosal. The meaning is not immediately apparent.

b. Perhaps the best assessment is to review the allowed daily intake of mercurials as calculated by Gerstner and Huff (1977) and to calculate, for comparative purpose, the mercury content of a single dose of thimerosal-preserved vaccine.

The calculations by Gerstner and Huff are for methyl mercury (that is perhaps 10 times as toxic as phenolic mercury).

The values are:

	Hg
Critical total body methyl mercury burden in an adult (160 lbs)	40 mg
Critical daily intake (considering a 70-day half-life with continuing turnover)	400 µg
A safety factor of 1 in 10 is judged to be needed so that:	
The methyl mercury daily intake limit would be	40 µg

c. The Swedish Commission on Evaluating the Toxicity of Mercury in fish (based on methyl mercury) gave a maximum daily intake in adults of: 30 µg

Markert states that the normal average daily intake of mercury in adults is: 10-20 µg

d. WHAT IS THE MERCURY CONTENT IN THIMEROSAL-PRESERVED VACCINES?

(1) Thimerosal is generally used at 1:10,000 dilution. About half the weight of thimerosal is mercury.

Therefore, there are 50 µg of mercury/1.0 ml dose.
25 µg of mercury/0.50 ml dose.

(2) Translating to body weight

For a 160 lb adult 1.0 ml = 0.3 µg Hg/lb. body wt.
For a 6 lb baby 0.5 ml = 4.0 µg Hg/lb. body wt.

e. PUTTING THIS INTO PERSPECTIVE.

For adults: The 50 µg of mercury in a single 1 ml dose is 1.7 times the Swedish daily allowance of 30 µg of mercury. We must take note that this allowance is based on the assumption that the total body burden has already reached the estimated 40 mg critical level.

For babies: The 25 µg of mercury in a single 0.5 ml dose and extrapolated to a 6 lb. baby would be 25X the adjusted Swedish daily allowance of 1.0 µg for a baby of that size. The total mercury burden in a baby is unknown but it has been stated that the blood level of a newborn may exceed that of the mother. If 8 doses of thimerosal-containing vaccine were given in the first 6 months of life (3 DPT, 2 HIB, and 3 Hepatitis B) the 200 µg of mercury given, say to an average size of 12 lbs., would be about 87X the Swedish daily allowance of 2.3 µg of mercury for a baby of that size.

When viewed in this way, the mercury load appears rather large. It will be recalled that phenyl mercury toxicity is only about 1/10 that of methyl mercury and it might be justifiable to correct these calculated numbers by a factor of 10.

PERSPECTIVE AND CONCLUSION.

It appears essentially impossible, based on current information, to ascertain whether thimerosal in vaccines constitutes or does not constitute a significant addition to the normal daily input of mercury from diverse sources. It is reasonable to conclude, however, that thimerosal should be removed from single-dose vials when it can be removed, especially where use in infants and young children is anticipated. This is based more on perception than on any data that would point to thimerosal as a real hazard. The costs for single-dose vials may be prohibitive for most of the world population where multiple puncture multidose vials are used. The ethical justification for continued use of thimerosal-preserved multidose vials in developing countries would be based on the greater importance of disease prevention than the real hazard from giving small amounts of mercury preservative for which there are no reliable standards of safety.

In planning for the future of thimerosal-containing product, it will be important:

a. To measure preservative adequacy and to consider use of 1:20,000 thimerosal rather than 1:10,000.

b. Combine as many vaccines as possible into a single-dose product so as to minimize the cumulative total mercury administered in multiple dosing.

c. Solution to the problem for PedvaxHIB might be to consider the dried antigen as a single dose (which is thimerosal-free) and to consider the alum-containing diluent also as a single dose. This would reconstitute as thimerosal-free single-dose PedvaxHIB.

d It might be worthwhile, also, to do precise pharmacologic studies to measure thimerosal mercury accumulation, excretion, toxicology, etc. in animal experiments. It could be that the Swedish-allowed daily mercury limit dosage is excessively below the real safety threshold. There are no proved instances of thimerosal toxicity in routine clinical use of thimerosal-preserved vaccines.

It is worthy of consideration to find another acceptable preservative. This has been pursued in the past in a number of laboratories and the chance for success in the near time frame would probably be very small. The State Serum Laboratory, Stockholm, has such a program just starting.

CBER (Sharon Risso) 2 years ago made a summary of preservatives used in biological products . Her stated list was:

Antibiotics:

Amphotercin B	-	Rabies
Kanamycin	-	?
Neomycin	-	Measles
Polymyxin	-	Influenza
Streptomycin	-	Live polio

Chemicals:

Benzalkonium Chloride	-	Anthrax
Thimerosal	-	Many
Phenol	-	Polysaccharides, typhoid, interferon
Formaldehyde plus 0.5% 2 phenoxyethanol	-	Killed polio
Formaldehyde	-	Mumps skin test

This gives no real choices for polypeptide/protein vaccines. It may be worthy of note that surfactants such as benzalkonium chloride release toxins from gram negative bacteria.

ces:

Markert, J.R. et al. Lymphocyte levels in subjects with and without amalgam restorations. JADA 122:49, 1991.

2. Mason, M.M. et al. Toxicology and carcinogenesis of various chemicals used in the preparation of vaccines. Clin. Toxic. 4:185, 1971.

3. Report of an International Committee. Maximum allowable concentrations of mercury compounds. Arch. Env. Health. 19:891, 1969.

4. Gerstner, H.B., et al. Clinical toxicology of mercury. J. Toxic. Env. Hlth. 2:491, 1977.

5. Merck Index, 1989.

6. Andersson, B. State Bact. Laboratories preliminary report.

7. Personal communications, Company memos, correspondence, textbooks, etc.

M.R.H. - 5532

P.S. The translation of a recent article on thimerosal preservative in vaccines by Dr. Hans Wigzell (copy appended) has just been received. Dr. Wigzell is the Director of the State Bacteriological Laboratories in Stockholm. The facts and considerations are consistent with those given in this memo.

The seasoned conclusion Wigzell gives is, "Our opinion, however, is that the problems associated with the spread of mercury via vaccination are so minor that there is no reason to push a hastened solution".

Note, however, that Wigzell mentions only thimerosal-preserved DTP or DT given in at least 3 doses since the 1950s. Even with such small exposure, Sweden is moving as expeditiously as feasible to achieve a zero input of mercury from thimerosal.

M.R.H.

Attachment - 1

cc R. Bennett, K. Brown, A. Elliott, R. Ellis, E. Fagan, P. Friedman, R. Goldberg, C. Henderson, C. Hildebrand, J. Ryan, J. Sandelands, E. Scolnick, J. Shafer

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