

C. R. BENNETT

MAR 27 1991 3/26/91

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

FILE _____
 FOLLOW UP _____
 CIRCULATE _____

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 (CDER) 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:

9244. Thimerosal. Ethyl(2-mercaptobenzoyl(2-)-O,S)-mercurate(1-) sodium; [(2-carboxyphenyl)thio]ethylmercury sodium salt; sodium ethylmercurithiosalicylate; thimerosal; mercurothiolate; Merthiolate; Merzonin; Merzogan; Merfamin. C₁₀H₉HgNaO₃S; mol wt 404.84. C 26.70%, H 2.24%, Hg 49.35%, Na 3.68%, O 7.90%, S 7.92%. Prep'd by reacting ethylmercuric chloride (or ethylmercuric hydrosulfide) with thiosalicylic acid; Kharasch. U.S. pat. 3,672,615 (1928); Trikojus. *Nature* 158, 472 (1946); Swirski et al. *Pharmazie* 39, 371 (1960). C.A. 55, 3307a (1961). Toxicity: Mason et al. *Clin. Toxicol* 4, 185 (1971).

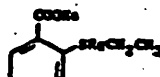
Stabilization of salts with EDTA: Davison. U.S. pat. 2,864,844 (1958 to Lilly). pH of 1% aq soln: 6.7. LD₅₀ a.c. in rats: 98 mg/kg (Mason).

USE: Pharmaceutical 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 5 ml alcohol. Practically insol in ether and benzene.



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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 include eczema, generalized exanthems and urticaria.

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- 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.

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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 LD₅₀ 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.

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(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.

7. 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.

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- 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.

Sources:

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

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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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Source: LAKARTIDNINGEN 57:621, 1990
(Translation from Swedish by Maria Pravt, Blue Bell
(3/26/91))

DIFFICULT TO SUBSTITUTE MERCURY AS A PRESERVATIVE IN BACTERIAL VACCINES

During the last couple of years The National Swedish Bacteriological Laboratory (SBL) has received an increasing number of inquiries regarding the presence of mercury in bacterial vaccines. We from SBL will give a brief account on some commonly asked questions with regard to this matter.

Thiomersal sodium (Merthiolate, ethyl-2-carboxyphenylmercapto mercury sodium) is an organic mercury compound, which is used in pharmacy as a preservative with antimicrobial effect. Thiomersal sodium is added at the end of vaccine preparation and is therefore not needed during the real manufacturing process. It keeps the vaccine preparation sterile during its use at the clinic and child health station. The vaccine is distributed in multiple dose vials. Vaccine extraction from the bottle therefore takes place several times and a risk of contamination exists.

Difficulties in adequately refrigerating the vaccine bottles during ongoing vaccination can also add to increased risk of bacterial growth in vaccine solution. Europapharmacopoeia demands that preservatives be added to biological preparations when distributed in multiple dose vials.

Vaccine preparations contain 0.1 mg thiomersal sodium per each ml of vaccine. About half the weight of thiomersal sodium is made up of mercury. When the volume of a dose duplex vaccine is 0.5 ml then the indicated amount of thiomersal sodium is 0.05 mg or 50 mcg, and mercury content is 0.025 mg or 25 mcg per vaccine dose.

VARYING CONTENT OF MERCURY IN OUR ENVIRONMENT

To explain the given amounts in relation to other mercury levels in our environment it can be mentioned that different investigations during the 1960s and 1970s showed that av daily diet contained 4-11 mcg organic mercury. Fish from the sea without direct contamination contains as a rule 10-50 mcg mercury/kg. A few fish species can however contain 1 mg/kg or more.

The content of mercury in the air in Sweden varies markedly. The inhaled quantity, expressed according to size, is 0.2 mcg/day and is mainly made up of mercury vapour.

For work environment, the Swedish Association for Industrial Safety limits the hygienic level for certain organic mercury compounds to 10 mcg/cu m and for other mercury compounds to 50 mcg/cu m air.

Organic mercury compounds are absorbed as complete from intestines and are mainly stored in the red blood cells. Excretion is mainly from liver via gallbladder.

Since the national inoculation against diphtheria/tetanus/pertussis (triple vaccine) began in the fifties, the majority of all children in Sweden have received at least 3 doses of the vaccine against either diphtheria/tetanus or diphtheria/tetanus/pertussis.

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These vaccines have always contained thiomersal sodium as a preservative. The vaccination-delivered amount of mercury can not be viewed as alarmingly high in relation to the above-mentioned amounts from other sources. A difference is however in the route of administration. There are however no investigations that show that there is a difference in general toxicity when the uptake of mercury is from the stomach-intestines or after injection.

LOCAL REACTIONS

Since the middle of 1970s, SBL has been aware that thiomersal sodium can cause local reactions. There are numerous short notices in the literature regarding observations of such reactions, even a number of reports on the frequency of reactions against thiomersal sodium in specific population groups. Local reactions are believed to be of allergic hypersensitive delayed-type ones. The frequency of such reactions vary depending on potential previous exposure to thiomersal sodium.

There are also reports of general reactions in patients treated with mercury-containing medications. This should be studied in relation to the tremendous large number of subjects vaccinated with preparations containing thiomersal sodium.

Lately we at SBL have tried to find ways to manufacture mercury-free preparations. In part we have tried to find substitutes for organic mercury compounds, in part we have tried to develop vaccine preparations completely free from preservatives.

DIFFICULTIES TO FIND SUBSTITUTE

Attempts to find substitutes for organic mercury have not been successful. Other preservatives which fulfill the pharmacological requirements to kill test organisms have proven to be unsuitable for other reasons - they increase human toxicity or affect vaccine preparations.

Attempts have been made at SBL to develop a mercury-free vaccine combination with polio, tetanus and diphtheria. The results of these studies have so far been satisfactory. With respect to tetanus and diphtheria several try-out series are being conducted to secure full safety with respect to eventual influence of vaccine properties.

The best way to go is to switch to dispensing the actual vaccines without adding preservatives. From the sterilization point of view it is possible to dispense the vaccine in single-dose vials without adding any preservatives. Equivalent multiple dose vials can also be made, but these must then be used up the same day as when the vaccine ampule has been broken. Therefore this is a cost consideration the head of health services has to consider. Several small ampules or bottles are more expensive than a smaller number of larger packages. The handling of the small packages is also more cumbersome at the time of vaccination.

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A PREMATURE SOLUTION IS NOT DESIRED

Our goal is to develop, as soon as possible, vaccines completely free of mercury. For the above-mentioned reasons it is concluded that this will, however, take a considerable length of time, because durability (shelf-life) studies are time consuming. In addition clinical testing has to be done. Our opinion however is that problems associated with the spread of mercury via vaccination are so minor that there is no reason to push a hastened solution.

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