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Thimerosal: Mercury metabolism and distribution in laboratory rats?

The most recent rat study (JL Rodriques et al. 2010[1] [Rodriques]), which compared the dosing of mercury from Thimerosal to the mercury from "*methylmercury*", used a more self-consistent "dosing" design than the 2005 study (TM Burbacher et al. 2005[2] [Burbacher]) in infant monkeys.

Unlike the 2005 Burbacher study, which injected the Thimerosal and gavaged (force-fed) the "methylmercury" compound studied, the 2010 Rodriques study force-fed solutions of both.

In addition, the Rodriques study more exactly determined the nature of the types of mercury compounds in the blood and tissue samples assessed than the Burbacher study did.

However, like the cited Burbacher study in infant monkeys, the 2010 Rodriques study suffers from some significant design and reporting weaknesses including, but not limited to:

1. This study inappropriately doses the mercury compounds by force-feeding them rather than, *as it should*, if it truly were a study interested in studying the comparative toxicokinetics of mercury compounds, injecting them into the rats subcutaneously[3].
2. Similar to an unsupported declaration made in the Burbacher study, the Rodriques study makes an unsubstantiated claim: "*Our studies demonstrate that mercury derived from TM*" [the abbreviation used for Thimerosal] "*is cleared from the rats more quickly than Met-Hg*"[4,5,6,7].

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1 Rodriques JL, Serpeloni JM, Batista BL, Souza S, Barbosa Jr F. Identification and distribution of mercury species in rat tissues following administration of Thimerosal or methyl mercury. Arch Toxicol 2010; 84: 891-896. [**Note:** In these studies, the compared methylmercury compound is methylmercury chloride, and both the aqueous Thimerosal and aqueous methylmercury chloride were force-fed.]

2 Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarksson T. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosal. Environ Health Perspect 2005 August; 113(8): 1015-1021. [**Note:** In these studies, the compared methylmercury compound was methylmercury hydroxide, and the Thimerosal solutions were injected while the "methylmercury" solutions were force-fed.]

3 This is a major experimental design flaw because: **a)** in vaccines and other parenteral drugs preserved with Thimerosal, the real-world use where there is a toxicity concern, the Thimerosal is injected and **b)** unless the doses is injected, **1)** there is no assurance that all of the dose is absorbed into the test animal, **2)** the observed redistribution will be a mixture of the kinetics of absorption into the body and the kinetics of redistribution, metabolism and excretion which is knowingly not representative of the toxicokinetics of the real-world situation, the kinetics of the metabolism, redistribution and excretion of Thimerosal injected into the human body. Since the figures in Burbacher, where the Thimerosal was injected (Burbacher Fig. 6) and the comparator methylmercury compound used was force fed (Burbacher, Fig. 2), clearly show patterns of absorption delay for the increase in the mercury level in the blood compared to the injected Thimerosal and Rodriques cites that study, there is no scientific justification for continuing to use force feeding as the exposure method.

4 Based on the authors', "*Moreover, just 6 h after administration, mercury is found mainly as inorganic mercury in TM-exposed animals (data not shown)*", the study actually only proved that the complex "Thimerosal" mixture that was force-fed to the rats was more rapidly concerted into the form of "inorganic" mercury than was the solution of methylmercury chloride that was force-fed to a second group of these rats.

5 Since the study presents no mass balance data showing where "100%" of the dosed mercury (Hg) ends up and does not report values for Hg in the urine and feces of the rats, the authors have not shown that the mercury clears the rats in 5 days.

6 In studies conducted in Japan by Yasushi Takeda et al. (see footnote 9) using two radiolabeled (^{203}Hg) ethylmercury compounds, ethylmercury chloride [EtHgCl] and ethylmercury cysteine [EtHgCys], at an injected "10 mg of mercury/kg" dosing level, the percentage of the doses excreted in the urine and the feces at 5 days post administration were apparently (from the cited article's Figure 3) only a small percentage of the mercury doses administered. For the EtHgCL, the total percentage of the dose excreted in 5 days was apparently about 10 % of the initial dose; for EtHgCys, that % was apparently less than 15% of the initial dose.

7 "*Met-Hg*" is the Rodriques article's shorthand for methylmercury-derived mercury compounds and "*TM*" is its abbreviation for "Thimerosal"-derived mercury compounds.

3. Like Burbacher, the "methylmercury" compound chosen, methylmercury chloride in the current Rodriques study (as compared to methylmercury hydroxide in Burbacher et al. 2005), was the wrong compound upon which to base truly comparative toxicological distribution evaluations[8,9].

4. The purity[10] of the Thimerosal used was not disclosed, and no data was presented that verified that the Thimerosal solutions used had the nominal level of "Thimerosal"-related mercury (Hg)[11] in them just before the rats were dosed with said "Thimerosal" solutions.

In addition, this study failed to monitor the blood Hg levels at:

- 30 minutes after administration and
- 1 hour after administration.

Based on the researchers' findings: "*... were rapidly absorbed*" and "*just 6 h after administration, mercury is found mainly as inorganic mercury in TM-exposed animals*", these earlier sampling times are clearly required for studies that force-feed or inject water-based solutions of Thimerosal (TM).

Thus, there are no baseline levels and no levels near the time of administration of the compounds to assess the peak level, and the initial absorption and distribution of the Hg into the rats' blood from the basis Hg-containing solutions administered.

Lacking data to verify the initial levels of "Thimerosal" Hg and the initial uptake of the two different Hg components (the complex Thimerosal-based mixture and the single-component methylmercury chloride solution), the blood levels in the Rodriques study's "*Fig. 1*" only show that, besides an apparent kinetics-related offset, the migration of the Hg species from the blood into the rats' tissues seems to be "similar" even though the analysis-result patterns for the Hg-containing substances quantified clearly differ.

The declaration, "*After the rats were exposed to TM or Met-Hg, both species were rapidly absorbed*" confirms: **a)** the Rodriques researchers were either: **1)** unaware of the instability of Thimerosal in aqueous solution or **2)** chose to ignore its instability and **b)** knew their tissue-distribution data was biased by the delaying effects of absorption from the gut into the body in the test rats studied.

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[8] Since Rodriques cites the Burbacher paper 5 times, this reviewer, having studied the Burbacher paper, finds it difficult to think that the inappropriateness of the choice of "Me-Hg" chloride as the toxicokinetic comparator was unrecognized.

[9] If the goal is, as it should be, to determine the difference in the distribution of an ethylmercury compound, Thimerosal in this instance, used as an active (preservative) in a drug (vaccine) and a corresponding methylmercury compound when it is known that the Thimerosal is not stable in a vaccine formulation but rather is, at any time after dissolving the Thimerosal in an aqueous saline mixture containing proteins and dissolved oxygen (a vaccine formulation), the solution is some mixture of Thimerosal, ethylmercury chloride, ethylmercury hydroxide, sodium thiosalicylate and the disulfide of sodium thiosalicylate (as revealed in the process patents [see, for example, Kharasch US 1672615 (1928)] for the manufacture of Thimerosal), then the methylmercury analog of Thimerosal should be used as the comparator. Absent the saline, the complex mixture is, of course, Thimerosal, ethylmercury hydroxide, sodium thiosalicylate and the disulfide of sodium thiosalicylate.

[10] Commercial samples of Thimerosal may contain a significant impurity level of some methylmercury impurity and this writer notes that the source cited, Sigma Chemical, St Louis, MO, seems to sell Thimerosal that is about 97% to 98 % pure without disclosing the nature of the major impurities.

[11] Since Thimerosal, unlike the inorganic mercury, methylmercury chloride and ethylmercury chloride compounds used, is not-at-all stable in aqueous solution (see the patent cited in Footnote 5), it is imperative that the actual level of mercury in solution and, *for speciated toxicokinetic studies*, the nature of the Hg species in the Thimerosal solution be determined just before it is administered to the test animals.]

Further, the authors report, "*Moreover, just 6 h after administration, mercury is found mainly as inorganic mercury in TM-exposed animals (data not shown)*", clearly indicates a much faster rate of metabolism for the Hg species in the rats given "Thimerosal" than for the Hg species in the rats force fed methylmercury chloride.

This finding also confirms the need in the Thimerosal-treated rats for initial blood sampling at much at shorter intervals.

The interesting commonalities between the Burbacher and Rodriquez studies include:

- The similar findings for relative levels of "inorganic Hg" species accumulating in the brain[12] of the two test groups of rats, with Rodriques finding about 2.2 times as much in the rats treated with Thimerosal on average as in those similarly treated with methyl-Hg chloride;
- The approximate (order of magnitude) levels of "inorganic" Hg in the brain between: **a)** the rats treated with a single 0.5 mg of Hg from "Thimerosal" dissolved in water/kg of rat body weight and sacrificed at 5 days post treatment (about 170 ng of "inorganic Hg"/g of rat brain on average) and **b)** the monkeys treated with 4 doses of 0.020 mg of "Thimerosal" in a vaccine [aqueous buffered saline] matrix or 0.08 mg of "Thimerosal" and sacrificed around 5 days (1 – 6 days) after the last dose of "Thimerosal" was administered (roughly 15 ng of "inorganic Hg"/g of brain on average at 22 to 27 days after initial dose was administered); and
- Both groups of researchers: **i)** focused on the Thimerosal-derived Hg species as being at a lower level at the time points where measurements were made than the methyl-mercury-derived Hg species and **ii)** claimed "*clearance*" (implying that the Thimerosal-derived-mercury species are clearing the animal) based solely on the average drop in the level of mercury in the animal's blood.

The interesting difference was the Rodriques study's finding of "methylmercury" in the "Thimerosal"-treated rats.

This finding indicates that either:

a. The pathway for the generation of the inorganic-Hg species present in the tissues proceeds through repetitive demethylation steps (Thimerosal ➔ simpler ethyl-Hg species ➔ simpler methyl-Hg species ➔ inorganic-Hg species)[13] or

b. *Less likely*, the ng/g levels of "inorganic" Hg species in the kidney (9,581.9 ng/g), liver (2999.7 ng/g), heart (844.7 ng/g) and the brain (162.9 ng/g) have been reduced by some sort of internal "methylation" reconversion of some of the inorganic-Hg species into methyl Hg species [kidney (95.9 ng/g), liver (71.9 ng/g), heart (89.7 ng/g) and the brain (61.3 ng/g).

Based on the failure to detect any ethyl-Hg species in the heart tissues tested, this reviewer thinks that hypothesis "**a**" is probably the valid one.

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[12] In the absence if any corrective treatment regimen, the adverse neurological symptoms observed in humans most often begin to develop after the last dose of a Thimerosal-preserved vaccine is administered, these adverse symptoms persist, and, in normal humans, the half-life of these "tissue entrained" inorganic mercury species in the human brain is about 2 decades (20 years). Thus, given the long-term effects and the bioaccumulation of Hg observed, it is important to focus on this inorganic Hg because, based on Rodriques, when Thimerosal was the study compound, more than 60 % of the mercury in the brain samples was in the form of inorganic mercury.

[13] Based on all of the data presented, hypothesis "**a)**" is the more likely one. Moreover, it is a hypothesis that is consistent with the data observed for the identified types of mercury in the heart samples tested.

This seems to be the case because "all" of the "Thimerosal"-related, ethyl-Hg species have apparently been reduced to mostly inorganic-Hg species (844.7 ng/g) with some remaining methyl-Hg species[14] (89.7 ng/g) in the Thimerosal-fed rats' heart tissues at 5 days after dosing.

Thus, at this point in time, the methyl-Hg species level in the heart (measured as MeHgCl) is roughly 11 % of the inorganic-Hg species level (quantitated as mercuric chloride [$HgCl_2$]).

A Review of the Blood Data

Returning to the "***Results***" narrative and focusing only on Thimerosal, the authors reported (with emphasis added):

> "*After the rats were exposed to TM or Met-Hg, both species were rapidly absorbed. Figure 1 shows the behavior of mercury in the blood of rats exposed to TM ... plotted against hours after dosing. As can be seen, the blood of rats exposed to TM always presented much lower levels of mercury than that of Met-Hg-exposed animals. On the other hand, the rate of decline in blood mercury was faster in rats exposed to TM. Moreover, just 6 h after administration, mercury is found mainly as inorganic mercury in TM-exposed animals (data not shown). .... After 48 h of mercury administration, most of the mercury in the blood of the TM-exposed rat was in the form of Ino-Hg with small fractions in the form of Met-Hg and Et-Hg. However, 120 h after exposure, neither Et-Hg nor Met-Hg was detected in the blood of TM-exposed rats. .... Brain concentrations of total Hg were three times lower for the TM-exposed animals. Similar levels of Hg in liver were found in both Met-Hg- and TM- exposed rats. On the other hand, kidney Hg levels were higher for the TM group. Moreover, mercury was mainly found as Ino-Hg in liver and kidney, corresponding to 83.9 and 95.3% of the total amount in liver and kidney tissue, respectively in the TM-exposed group. Interestingly, of the total amount of brain mercury in these same animals, a significant fraction (23.7%) was found as Met-Hg, and 13.5% was in the form of Et-Hg. ....*"

Based on the reported data for Thimerosal (including rapid absorption and complete conversion in the blood into inorganic-Hg species by 120 hours [the time at which the test rats were sacrificed]) and the general relation that "complete" metabolism of a species can be defined as 6.6 times the half-life of the species, the half-life of "Thimerosal"-related ethyl-Hg species dosed in the blood can be estimated as being $\leq$ 120 hours/6.6 or $\leq$ 18 hours.

Presuming roughly first-order metabolic kinetics, the peak Thimerosal-derived-Hg concentration can be estimated as about twice the concentration at 18 hours (about 400 ng of Hg/mL based on the data in "*Fig. 1*" [Rodriques, page 894]) or ~ 800 ng of Hg/mL (i.e., roughly 800 ng Hg/g of blood presuming the rats' average blood density was about 1 g/mL).

Since the initial Hg dose was 0.5 mg/kg (or 500 ng Hg/g of rat weight), clearly, the estimated peak concentration of Thimerosal-derived Hg is about 1.6 times what the average concentration in the rat would have been, presuming the mercury dosed was uniformly distributed[15].

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[14] Given the many compounds in the human body with which any and all alkylmercury compounds may interact or react, this reviewer cautions the reader to avoid the logical trap that the measurement of discrete inorganic, methyl and ethyl mercury compounds after sample work-up is simply a matrix isolation effect when, *given the conditions of the sample work-up*, the sample work-up *actively* converts "all" of the various mercury species in the sample into the three compounds that are then measured.

[15] Since Yasushi Takeda et al. (Takeda Y, Kunugi T, Hinsino O, Ukita T. Distribution of Inorganic, Aryl, and Alkyl Mercury Compounds. ***Toxicol Applied Pharmacol*** 1968; **13**: 156-164) reported (see: that article, bottom of page 158, "*Total amount of*

Given the finding that "*just 6 h after administration, mercury*" [in the rats' blood] "*is found mainly as inorganic mercury in TM-exposed animals*", it appears that more than half of the Thimerosal-derived Hg in the rats blood has already been completely metabolized – indicating that the metabolic blood half-life for the dosed alkyl Hg species (Thimerosal and its "metabolic" breakdown alkyl-Hg-containing components) is actually less than 6 hours.

At "*120 h after exposure, neither Et-Hg nor Met-Hg was detected in the blood of TM-exposed rats*" and the apparent level of "inorganic Hg" species probably distributed at different levels in the different major components within the blood (simplistically, the plasma, the red blood cells and the white blood cells) is ~ 45 ng per mL (or g) of blood.

Since no evidence is presented that the rats excreted a significant percentage of the initial dose in their urine and/or feces in the 5-days after dosing, this reviewer must presume (see footnote **6**) that probably 85 or more percent of the initial dose has left the blood and now resides in the rats' tissues in a variety of chemical forms.

A Review of the Data for Thimerosal in the Blood and Tissues

Table 1r Forms of mercury ~~identified~~ quantified in tissues of rats exposed to [T]himerosal* or methylmercury chloride [with revisions of the Rodriques table in red and additions to it in blue]

	Form of mercury ~~identified~~ quantified									**Total Hg**
	Ino-Hg			**Met-Hg**			**Et-Hg**			
	Concentration found (ng/g)	SD	% Ino-Hg	Concentration found (ng/g)	SD	% Met-Hg	Concentration found (ng/g)	SD	% Et-Hg	Concentration found (ng/g) **
Thimerosal (n = 5)										
Brain	162.9	11.7	62.8	61.3	1.1	23.7	35.0	1.1	13.5	259.2 [0.52]
Liver	2999.7	62.3	83.9	71.9	4.1	2.0	503.1	68.9	14.1	3 574.7 [7.1]
Heart	844.7	37.3	90.4	89.7	2.3	9.6	ND	ND	ND	934.4 [1.9]
Kidney	9581.9	164.3	95.3	95.9	31.4	0.9	382.2	18.4	3.8	10 060.0 [20.1]
Inorganic Hg in blood***	~ 45.0	---	---	'none'	---	---	'none'	---	---	~ 45 [0.09]
Initial dose	---	---	---	---	---	---	"500.0"	---	---	-----

* Throughout this article Thimerosal, a trade name for sodium ethylmercury thiosalicylate, has been capitalized except when the original article was quoted because American English classifies trade names as proper nouns, which should be capitalized.

** Numbers in brackets [ ] are the total Hg in ng/g found divided by the initial "500 ng/g" dose[16].

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blood and muscle were assumed to be 5 and 33 of the body weight, respectively") that blood only makes up about 5% of a rat's weight, this estimate of "~ 800 ng of mercury/g of blood" level for the mercury in the blood translates into a ~ 40 ng/g of body weight contribution or only bout 8% of the initial dose of 500 ng of mercury/g of body weight. Similarly, the 120-hour, "~ 45 ng of mercury/g of blood" level for mercury translates to a level of inorganic mercury species in the blood that is less than 0.5% of the initial dose. [**Note:** Based on the reported much slower conversion of EtHgCl into "inorganic" mercury and the levels of mercury reported, its "maximum" relative mercury level in blood can also be graphically "estimated" to be similar to the Thimerosal's estimated maximum mercury level.]

16 Yasushi Takeda et al. (Takeda Y, Kunugi T, Hinsino O, Ukita T. Distribution of Inorganic, Aryl, and Alkyl Mercury Compounds. ***Toxicol Applied Pharmacol*** 1968; **13**: 156-164) reported on the distribution patterns in rats for two appropriately radiolabeled (^{203}Hg) ethylmercury compounds, ethylmercury chloride [EtHgCl] and ethylmercury cysteine [EtHgCys], dosed subcutaneously at the level of 10-mg of mercury per kg of body mass and, without speciation, the total levels of mercury exposure were tracked in the blood, kidney, liver, spleen, muscle and brain. The patterns of mercury distribution relative to the dose in the like tissues studied (based on the data reported at 1, 2, 4 and 8 days after injection of the ethylmercury compounds) were *crudely* similar in the like tissues (brain, liver and kidney; 0.145 and 0.202 for EtHgCL and EtHgCys respectively in the brain , 1.51 and 1.74 in the liver, and 7.05 and 9.51 in the kidney) at 4 days (to those computed from the data reported in Rodriques at 5 days for Thimerosal [0.52 for the tissue level to dose level in the brain, 7.1 in the liver, and 20.1 in the kidney)

*** Estimated from the data in Rodriques "***Fig. 1***" at "*120*" hours" (5 days).

For the Thimerosal-derived-Hg species (see **Table 1r**, revised from Rodriques "***Table 1***"), this reviewer wishes to make the following observations:

- Unfortunately, the Rodriques study did not provide the weights of the rats and the weights of the various organs tested so that this reviewer could compute the percentage of the applied mercury dose in each of the organs at 5 days post exposure.
- The word "*identified*" in "***Table 1***" of the Rodriques article should have been replaced with the word "quantified" because the sample-work-up procedure used converts the mercury present into the discrete "chloride" components that are then quantified by comparison of their response to the responses observed for various levels of 3 standard Hg-containing compounds: mercuric chloride ($HgCl_2$), methyl-Hg Chloride (H_3CHgCl) and ethyl-Hg chloride ($H_3C\text{-}H_2C\text{-}HgCl$).
- Based on the initial dosing of the Thimerosal at the level of 0.5 mg of Hg/kg of rat body weight, this reviewer notes that, after 5 days:
  - The mean level of mercury (Hg) in the brain is more than 50% of the administered level of Hg (restated as 500 ng Hg per "g" of rat weight, for ease of comparison to the tissue levels),
  - The mean level of Hg in the liver is more than 7 times the administered level of Hg,
  - The mean level of Hg in the heart is about twice the administered level,
  - The mean level of Hg in the kidney is more than 20 times the administered level, and
  - The mean level of Hg in the blood is less that 10% of the administered level.
- Presuming the conversion of Thimerosal (and the ethylmercury species into which it is converted in the body) into the Thimerosal-derived "inorganic mercury" species proceeds by a successive demethylation mechanism of some sort, then, *in the heart*, the "ethyl" to "methyl" conversion step has exhausted its "ethyl"-Hg substrates.
- Since the researchers report that there are no ethyl- or methyl- Hg species in the Thimerosal-treated rats blood after 5 days and, relative to the concentration administered, there are measureable levels of ethyl- and/or methyl- mercury species in the organs tested, especially in the Thimerosal-treated rats' liver and the kidneys, these alkyl-Hg species would seem to be strongly bound in these tissues and, therefore, may differ chemically from the Thimerosal-derived-Hg species force fed to the rats initially.

Thus, this paper clearly establishes that Thimerosal-derived Hg preferentially bioaccumulates in various tissues where, *in "normal" humans (based on other studies)*, its half-life is, *depending on the tissue of interest*, about one to two decades (10 to 20 years).

However, though the analysis method used in Rodriques permits the direct quantification of the mercury present in the blood and tissues as "inorganic mercury" (probably mercuric chloride), "methylmercury chloride" and "ethylmercury chloride", it does not identify the exact chemical nature of the mercury components in the tissue before the work-up used is initiated.

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even though the dosing levels were 20 times higher in the 1968 study. However, the ratios for the total level of mercury in the blood at 4 days to the dosing level in the 1968 studies (3.31 and 5.53 for EtHgCl and EtHgCys, respectively) were significantly higher than the about 0.1 ratio estimated from the graph in **Figure 1** at 5 days for total blood mercury in the Thimerosal dosed.

Further, the comparative values it reports relative to some standard levels do not necessarily mean that all of the mercury reported as a given mercury component (for example, "*methymercury*") was a single mercury species of some type before the sample was worked up for analysis.

In addition, based on studies using radiolabeled (^{203}Hg) compounds, Japanese researchers have shown that, *as compared to monkeys*, the rat is much more resistant to bioaccumulation of mercury in its brain than the monkey.

From the radiographs of longitudinal cross sections of the rats that this reviewer has observed, the relative distribution of "organic mercury" in the monkey, *which is presumed to be similar to the distribution of mercury in humans*, is more uniform across the body and accumulates more rapidly and to a greater degree in the brain than it does in the rat[17].

Based on the finding reported in Rodriques, "*it appears that the toxicokinetics of TM*" [Thimerosal] "*is completely different from that of Met-Hg*" [methylmercury chloride], these researchers have confirmed that the "*toxicokinetics*" of these two substances are different.

Based on the data disclosed, the Thimerosal-derived mercury species were much more rapidly converted into inorganic mercury than the methylmercury chloride (MeHgCl) compound studied to assess how well its degradation and distribution tracked the results found for Thimerosal.

In addition, the significantly higher blood levels of mercury in the rats dosed with the MeHgCl points to a less rapid clearance of the MeHgCl from the blood, which, *based on the tissue data*, translates into tissue ratio levels for the "inorganic mercury" species in the organs studied (brain, liver, heart and kidneys) that are, *on average*, 0.449, 0.172, 0.239 and 0.157 fraction of the corresponding levels in the respective mercury species in the "Thimerosal"-solution-dosed rats.

For the organic-mercury-containing species, the corresponding average MeHgCl-related to Thimerosal-related organic-mercury ratios are 7.83, 6.30, 18.0, and 11.9 for the brain, liver, heart and kidneys respectively.

Based on these realities, this reviewer must agree, "*Met-Hg is not an appropriate reference for assessing the risk from exposure to TM-derived Hg*".

The Thimerosal Toxicokinetic Studies

Clearly, the studies by Burbacher and by Rodriques both support the inappropriateness of the study of any compound other than Thimerosal when the goal is to understand Thimerosal's toxicokinetics.

Hopefully, rather than investing further effort in other such comparative studies, these researchers and others will invest their experimental time and dollars in studies that directly assess the toxicokinetics of Thimerosal at low doses in male[18] animals from variety of mammalian models (e.g., the rat, the miniature pig, and the monkey).

In these studies, the researchers should inject appropriately ^{203}Hg-labelled Thimerosal dissolved in a vaccine-like solution in a four-armed study encompassing the period from gestation until at least half of the animal's nominal lifespan, where:

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[17] Takahashi T, Kimura T, Sato Y, Shiraki H, Ukita T. Time-dependent distribution of ^{203}Hg-mercury compounds in rat and monkey as studied by whole body autoradiography. ***J Hyg Chem*** 1971; **17**: 93–107.

[18] Only the males should be studied because studies have shown that their susceptibility to being harmed by the administration of mercury compounds at a given dose level is several time that of the females.

- In the first arm, the pattern and mode of administration for the injected Thimerosal should conform, *in relative developmental time*, to, and be patterned after, the CDC-recommended early childhood vaccination program that existed in the USA in 2000 and/or the similar programs that exist in the many developing countries today;
- In the second arm, the pattern and mode of administration will be the same as in the first arm except that a true placebo (buffered isotonic saline containing sugar and sodium thiosalicylate matching the vaccine's amount of Thimerosal added) should be administered at each scheduled time,
- In the third arm, the pattern and mode of administration for the injected Thimerosal should conform, *in relative developmental time*, to, and be patterned after, the CDC-recommended "lifetime" program that existed during the period from August 2009 through June 2010 in the USA[19], and
- In the fourth arm, the pattern and mode of administration will be the same as in the third arm except that a true placebo (buffered isotonic saline containing sugar and sodium thiosalicylate matching the vaccine's amount of Thimerosal) should be administered at each scheduled time.

Hopefully, these "injected Thimerosal in vaccine-like solution" studies will, at a minimum, collect blood samples at "0", 30 minutes, 1 hr, 4 hours, 12 hours, 24 hours, and, *where the relative developmental time interval permits*, as many additional days as needed for the blood level of mercury in the test animals to decline to half of its peak level after each dose of mercury is administered as well as collect the urine and feces excreted each day and appropriately measure the mercury levels and monitor the behaviors exhibited by all of the animals.

To assess the nature of the mercury levels in the animals' organs, 20% of the animals in each arm of the study should be sacrificed at 0.25, 0.5, 0.75, 1.0 and 1.5 times the dosing regimen's elapsed time.

Then, *using the methodology in Rodriques et al.*, the nature of the mercury species in each tissue (at a minimum, in Blood, Kidney, Liver, Spleen, Muscle, and Brain) should be assessed along with the appropriate counting of the radiolabeled mercury (^{203}Hg) to determine non-destructively (or, if that is <u>not</u> feasible, destructively) a "true" total Hg level to assess how well the total from the speciated analyses matches the total from the radiolabeled mercury.

Provided the types of mercury in each of the samples are appropriately separated and assessed, then, *in about 1 year for studies using the rat to about 3 years when monkeys are used*, all of the needed data (on mercury levels, mercury excretion patterns, and, critically, the behaviors of the developing animals – test and control) should be available to address not only the toxicokinetic

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[19] The cited 2009-2010 US vaccination program's recommendations permitted: **a)** 2 Thimerosal-preserved flu shots to be administered to pregnant women at "anytime" in pregnancy; **b)** 2 half-dose (0.25 mL) flu shots to be given to 6-month-old babies; **c)** 2 half-dose flu shots to be given to 7-month-old babies; **d)** annual half-dose flu shots for children each year until they turn 3 years of age; and **e)** full-dose flu shots annually every year thereafter until the child was 18 years of age or older. To simplify the experiments and have some chance that the developing fetuses will survive their mother's being vaccinated while carrying them, this reviewer suggests postponing the initial in utero vaccinations until the animal's pregnancy reaches 80% of the normal gestation period. To allow for some losses of fetuses, the initial number of pregnant animals with apparently healthy pregnancies should be at least 33 % larger than needed for the rest of the study. For the rat, this reviewer suggests not less than 45 male pups, for the miniature pig, not less than 24 male piglets will be needed, and, for the Macaque monkey, not less than 15 male infant monkeys will be needed for the test arms. In each animal model, similar numbers of female test and control subjects should be studied in a parallel study to 2000 US childhood vaccination schedule to assess the sex-related differences, if any, between the distribution and excretion patterns seen. For the control arms, there should be little need for an excess of control subjects and the numbers in each arm could be half those needed in the test arms.

realities but also the types of subacute mercury poisoning symptoms that the test animals developed and continued to exhibit until they are sacrificed.

In conclusion, instead of doing more less-than-useful comparative distribution-within-the-animal studies, researchers should undertake the requisite toxicokinetic studies for injected Thimerosal in a manner that **a)** establishes true mass balance, **b)** tracks the nature of the species that are formed and **c)** *critically* assesses the adverse behavioral effects, *if any*, that appear to develop: **1)** after any injection time point, **2)** persist and worsen over time and **3)** exist after the repeated injection of Thimerosal according to some pertinent defined schedule such as the ones proposed.

Finally, this reviewer would like to thank the Rodriques researchers for apparently establishing that the metabolism of Thimerosal into the inorganic mercury species that persist in the brain probably proceeds through successive demethylation stages in which tissue-entrained ethyl mercury species are converted into tissue-entrained methylmercury species and the tissues-entrained methylmercury species are then converted to tissue-entrained inorganic mercury species.

About this reviewer, Paul G. King, PhD

Paul G. King, PhD Analytical Chemist, is a scientist who has:

- ❖ *Intensively studied:*
  - *the use of mercury compounds in medicine,*
  - *vaccines and*
  - *vaccination programs*

  for more than a decade and
- ❖ *Sorted out the underlying science to the extent that he could find such from all of the published information available from those with differing views about the use of mercury compounds in medicine, vaccination and vaccination programs.*

In the area of Thimerosal, its toxicity, and its use as a preservative in drug products including vaccines, Dr. King understands the issues and has clearly established that:

- ❖ *The current "approved" Thimerosal-preserved vaccines are deemed to be adulterated drugs (under 21 U.S.C. § 351(a)(2)(B)) because the manufacturers of these vaccines have failed to prove that the preservative level of Thimerosal in each dose of vaccine is "sufficiently nontoxic …" as they have been required to since the late 1960s under the regulations:* ***a)*** *issued by the National Institutes of health and* ***b)****, when the responsibility for regulating vaccines was transferred to the US Food and Drug Administration, recodified in 21 CFR § 610.15(a) since 1973, and,*
- ❖ *Because the vaccine makers have failed to conduct and submit the requisite Thimerosal toxicological safety studies proving the level of Thimerosal in their Thimerosal-preserved vaccines is "sufficiently nontoxic …", the FDA administrators have illegally licensed said drugs since 1979 (see 21 CFR § 601.4(s)) when the current good manufacturing practice regulations were amended to require the administrators to have proof of all of the requisite safety requirements, including the "sufficiently nontoxic …" requirement that they and the makers of said Thimerosal-preserved vaccines have admitted have never been met (see the 2003 Congressional Report: "Mercury in Medicine – Taking Unnecessary Risks"..*

Furthermore, Dr. King has: ***a)*** *thoroughly reviewed much of the literature bearing on the toxicity of Thimerosal and its carcinogenicity, mutagenicity, and teratogenicity as well as its immune-system disruption at levels below 1 part per million (< 0.0001 %) and* ***b)*** *based on a paper recognized by the FDA (Mason et al. 1971[i]), determined that, for injected Thimerosal, the NOAEL (no observed adverse-effect level) in developing humans is less than (<) 0.0042 µg of mercury (< 0.0086 µg of Thimerosal) per kg of body weight per day – rendering a single 0.5-mL dose of most Thimerosal-preserved vaccines, which nominally contain 100 µg of Thimerosal per mL of vaccine, "toxic" (or, more precisely, not "sufficiently nontoxic …"), when injected into a pregnant woman, unless the pregnant woman and the child she is carrying together weigh significantly more than 3300 kg (7,275 pounds)[ii].*

For a detailed article on the determination of the approximate NOAEL injected Thimerosal for adult humans and developing children, the reader is encourage to read:

http://mercury-freedrugs.org/docs/090812_fnldrft_TheTruthAboutTheToxicityOfThimerosalr5b.pdf.

If any, after reading this review, the cited article or any other article published by this reviewer, you find any significant error for which there is unbiased science that clearly supports your alternative view, then, by all means, send your alternative view and the supporting toxicological documentation to me through dr-king@gti.net and, if your studies are truly unbiased, this author will be glad to: ***a)*** *modify his views accordingly and* ***b)*** *publish an updated article reflecting his modified views and crediting you and the unbiased supporting documents you submit.*

If you find areas where the text in this review has grammatical, spelling or word-usage errors, please let the author know so that he may appropriately correct them and publish an appropriately revised version of this article.

For additional information about Dr. King, his interests and his writings, the reader can visit the Internet web site, http://www.dr-king.com/.

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[i] Mason MM, Cate CC, Baker J. TOXICOLOGY AND CARCINOGENESIS OF VARIOUS CHEMICALS USED IN THE PREPARATION OF VACCINES. ***Clinical Toxicology***, 1971; **4**(2): 185-204.

[ii] This totally hypothetical example presumes the child in utero is exposed to half of the dose (or 12.5 µg of Hg), the mother carrying the child is exposed to half of the dose (or 12.5 µg of Hg), and the NOAELs for injected Thimerosal are: **a)** < 0.042 µg of mercury (Hg) per kg of body weight per day for the mother and **b)** < 0.0042 µg of mercury (Hg) per kg of body weight per day for the child developing in utero. [**Note:** Since human weights rarely exceed 1,000 pounds (454 kg), it should be obvious that the preservative level of Thimerosal is not safe to inject into a pregnant woman. Moreover, presuming that any vaccine must be safe ("nontoxic") to inject into child weighing roughly 3 kg, the safe level of Thimerosal in a vaccine is one that contains less than 0.0042 µg of mercury per kg divided by 3kg of baby weight or < 0.0014 µg of mercury per dose (or < 1.4 nanogram (ng) of Thimerosal per dose). Thus, none of the currently approved Thimerosal-preserved and reduced-Thimerosal vaccines is safe to be given to any developing child. Moreover, for vaccines restricted to adults, a "reduced Thimerosal" vaccine containing up to "1" µg of mercury per 0.5-mL dose is only "nontoxic" when an adult, who is not pregnant, weighs significantly more than 23.8 kg (52 pounds) and may only be "sufficiently nontoxic …" when a mercury-poisoning-susceptible adult weighs significantly more than 238 kg (525 pounds) .]