

**Monsanto**

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FROM  
(NAME-LOCATION-PHONE)

G. J. Levinskas - G2WF (4-8809)

DATE

July 19, 1984

CC:

G. Roush - G2WG

SUBJECT

REFERENCE

TO

A. M. Ford - G3WD

W. J. McCarville - G3WG

I have read through the three volumes of Dr. Silbergeld's deposition which you provided on Monday. I have dictated comments relating to several sections of those depositions. These have been typed, and I presume you have received them by now.

In some places, she had made very specific statements about various compounds which I would question. However, I have not had time to go back and check specific sources to ascertain whether or not they are accurate. If you desire, I will proceed to do so in consort with other staff toxicologists. Kindly let me know whether this would be a worthwhile exercise or whether the need for such action has passed.

*George J. Levinskas /STE*  
G. J. Levinskas

/dkr

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Page 399, line 12 - Cyanosis is not the accumulation of cyanides in the body because of lack of oxygen. Cyanosis is a blue coloration of the skin which results from an inability of tissue enzymes to obtain oxygen. The body has a fairly effective system for removing cyanide by converting it to phytol cyanate. That is a two detoxification mechanism since phytol cyanate is about 1/200th or 1/300th as toxic as cyanide itself. In acute cyanide intoxication, death results from anoxia because the body's mechanism for conversion of cyanide to phytol cyanate is exceeded. She compounds that error on line 20. The body does not overproduce metabolic cyanides in analine poisoning.

Page 446, line 20 - The implication is that despite the fact that \$30 million has been spent, dioxin is still present in Times Beach. While the commitment has been made to buy out Times Beach at a cost of over \$30 million, nothing like that sum has yet been spent. Certainly, that sum has not been spent on attempts at removal or cleanup of dioxin contaminated soil.

Page 465, line 11 - The references made to animal studies using pure 2,4,5-T. In the March 22 deposition on page 254, line 9, she questions whether any of these studies purported to have used pure 2,4,5-T actually were using pure material. One ought to declare and hold a consistent position. One should not accept the claim of purity, and then contest the claim of purity according to the points one wishes to make.

Page 468, line 1 - She is not convinced that TCDD is much of a mutagen. However, in the March 21 deposition, page 118, line 4, she is attempting to implicate TCDD in genetic damage. Mutagens damage genes. If TCDD is not much of a mutagen, how can it produce those transmittable, non-hereditary diseases, that she discusses on this and the following page.

Page 472, line 13 - The determination of causation is not a statistical exercise. Application of a chemical to skin or eye will determine whether the material is an irritant or corrosive. No statistics are necessary. If a chemical is fed to animals, and they all develop cancer, no statistics are necessary. The rigorous mathematical formula that she describes are used to determine whether observations are likely to have occurred by chance or not. Having determined the probability that these events may or may not have occurred by chance, the experimenter then makes a decision as to whether or not the observed effect was produced by the chemical. In other words, statistical analysis is an aid to making decisions. Decisions should not be made on the results of statistical analysis alone. Unfortunately, there is a current vogue, particularly with respect to risk assessment of carcinogens, to accept these statistical result as a final, definitive, invariably reliable conclusion.

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Page 473, line 13 - The approach she describes here is good. In arriving at a conclusion as to what may have caused an effect, every effort should be made to rule out all possible causes. Does she do this when she "critically reevaluates" data. As mentioned in earlier instances, does she apply that same rigid standard to everything she reviews, or does she selectively abstract those points which support her view or accept data she considers favorable without subjecting it to such scrutiny?

Page 509, line 16 - The catalog of differences and similarities in the responses of different species to dioxin can be compared to that appearing on page 333, line 1. There are some differences between those two sections.

Page 510, line 3 - A statement is made that it would be very difficult to generalize about neurologic effects because these have not been very systematically studied accross species. Since the majority of the dioxin work has been in rats and mice, the adequacy of testing accross species for the various effects could be explored.

Page 510, line 13 - She is greatly over simplifying the problem of extrapolating from animals to man. In fact, she make it sound more like a science than the art it is. After stating that the mechanism of the reactions is known and similar in all animals, including man, she states on line 19 that the only remaining issue is does dioxin get to those mechanisms in humans as it does in animals.

Page 512, line 1 - She states that the sensitivity of the guinea pig appears to be related to the distribution of dioxin. The question is how does one determine the distribution or, as she says on line 6 of this page, the "differences of internal dose" between animals and man. Earlier, on page 300, line 8, she notes that the biological half-life can be determined from toxical kinetics in a number of animal species. Those curves can be used for predicting effects in man. However, while the curves can be used to make the prediction, what is the mechanism by which one can test the validity of the predictions? In other words, while people do manipulate numbers, make extrapolations, etc., and they may even put faith in the conclusions that are reached from those manipulations, there should be some basis for testing or verifying in some fashion, the validity of the conclusions which have been drawn.

Page 512, line 18 - Guinea pigs are more sensitive to TCDD than mice or rats. They are at least one, possibly even two orders of magnitude more sensitive. I am surprised that she is not sure of that fact because the extreme sensitivity of the guinea pig to TCDD is frequently cited in the lay press as well as in technical journals.

Page 516 - line 8 - Parathion inhibits the enzyme acetylcholinesterase. That enzyme inhibits these cholinesterase which is the chemical messenger between nerve connections and nerve muscle connections. Thus, when cholinesterase is inhibited, the effect is similar to repetitive stimulation of a nerve. The net effect of this would be muscle tremors, and in severe cases, convulsions. It does not produce muscular paralysis.

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Page 516, line 20 - The effect on peripheral nerves will last as long as the parathion is present. In organic phosphate poisoning, such as parathion, the signs of intoxication, including the effect on nerves, appears relatively shortly after exposure. Depending on the dose, it may be rather rapid or it may be delayed as long as 24 hours. Upon removal from exposure to parathion, recovery will ensue in 24-48 hours. Any toxicologist familiar with organic phosphate pesticides could testify on this subject. Dr. Wayland Hayes at Vanderbilt would be an excellent choice for this.

Page 517, line 6 - Parathion does not produce a delayed neurotoxic action in the same sense as triorthocresyl phosphate, the classic example of this neurological condition.

Page 520, line 7 - If chlorinated phenols can denature membranes, then would they not denature the skin before they got into the body to denature nerve membranes? In other words, can one have serious systemic effects from chlorophenols without first having had severe, or at least detectable, external skin injury?

Page 521, line 6 - Halowax is a chlorinate naphthalene. I do not see how it can form dioxin. This a point which the chemists can describe better than I.

Page 533, line 10 - Just a reminder that she is expressing her "opinions on dioxin".

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1 . yes.

P 300

2 Q. You can't take the amount, the dose with  
3 these mice and in any fashion extrapolate that to man?

4 A. That's not what I'm saying, Mr. Love. That  
5 one dose, that one experiment which we discussed, in a  
6 totally different context I might state for the record,  
7 by itself, has relatively little to contribute to what  
8 we are presently talking about. People have done  
9 specific studies to measure the toxicokinetics,  
10 including the biological half-life which is a derived  
11 number from toxicokinetics, of TCDD in a number of  
12 animal species. Taking those curves, there are  
13 methods for relating those on a predictive basis to  
14 humans.

15 Q. But you have not done that.

16 A. That's not my interest in TCDD. Many people  
17 have done it. It is contained at length in the EPA  
18 document on TCDD, the Canadian government's document  
19 on TCDD. There is no need for me to do it.

20 Q. Is there any reason why you cannot testify  
21 about it?

22 A. I would be happy to testify about it. But

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Principal Investigator: Dr. E.E. McConnell

Collaborators: Ms. M. Harris, Dr. P. Albro, Dr. R. Hass

Objective: To determine bioavailability of dioxin in Missouri soil samples.

Species: Guinea pigs, Hartley, male, 200-250 grams, 6 animals/group.

Experimental Groups:

1. 2,3,7,8-TCDD in corn oil, 1 $\mu$ g/kg bw, volume 0.1ml/100g bw
2. 2,3,7,8-TCDD in corn oil, 3 $\mu$ g/kg bw
3. Corn oil control - vol 0.1ml/100g bw
4. Times Beach contaminated soil - quantity to equal 1 $\mu$ g TCDD/kg bw
5. Times Beach contaminated soil - quantity to equal 3 $\mu$ g TCDD/kg bw
6. Times Beach contaminated soil - quantity to equal 10 $\mu$ g TCDD/kg bw
7. Minker site contaminated soil - quantity to equal 1 $\mu$ g TCDD/kg bw
8. Minker site contaminated soil - quantity to equal 3 $\mu$ g TCDD/kg bw
9. Minker site contaminated soil - quantity to equal 10 $\mu$ g TCDD/kg bw
10. Uncontaminated soil - weight equal to amount used in Group 6
11. Uncontaminated Missouri soil spiked with 2,3,7,8-TCDD, 0.8 $\mu$ g/gram soil, quantity administered to equal 10 $\mu$ g TCDD/kg bw, Group 6
12. Red clay soil sample spiked with 2,3,7,8-TCDD, 0.8 $\mu$ g/gram soil, quantity administered to equal 10 $\mu$ g TCDD/kg bw
13. Sandy soil sample spiked with 2,3,7,8-TCDD, 0.8 $\mu$ g/gram soil, quantity administered to equal 10 $\mu$ g TCDD/kg bw
14. Loam soil sample spiked with 2,3,7,8-TCDD 0.8 $\mu$ g/gram soil, quantity administered to equal 10 $\mu$ g TCDD/kg bw.

Experimental Design: Guinea pigs will be allowed to acclimate for 6-7 days after arrival at NIEHS. Guinea pigs will be individually housed in disposable cages (45x20x20cm) with food and water available ad libitum. One day prior to dosing, animals will be transferred to the high hazard animal facility, Room 1421. \*All animals will be lightly anesthetized with Metafane.

\* Guinea pigs will be weighed and randomly assigned to experimental groups. Food will be withheld for 24 hours prior to dosing.

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Those receiving TCDD in corn oil and corn oil alone will be gavaged using a 20 gauge 1 and 1/2 inch stainless steel needle. Guinea pigs receiving soil samples will be intubated using polyethylene tubing, PE190.

Animals will remain in high hazard room for a minimum of 48 hours before being transferred back to Bldg. 15 animal facility. Guinea pigs will be observed daily and weighed three times per week for 30 days.

Parameters Evaluated:    Body weight changes  
                                 Organ weights  
                                 Histopathology  
                                 Deaths

On day 30, guinea pigs will be weighed and euthanized using carbon dioxide. Animals will be observed for any gross changes with organs in situ. The following organs will be removed and weighed and fixed in NBF, 10%, for subsequent histopathologic evaluation: brain, thymus, spleen, right kidney, liver and right testicle. These organs will be removed and fixed in NBF, 10%, for subsequent histopathology: liver/gall bladder, thymus, spleen, left kidney, urinary bladder, stomach and left testicle.

Any animals becoming moribund or dying during the 30 day experimental period will be weighed and opened, observing organs in situ. Tissues listed above will be fixed in Neutral Buffered Formalin, 10%, for subsequent histopathology.

Preliminary analyses of Missouri soil samples by Analytical Chemistry Group indicate ~ 600ppb 2,3,7,8-TCDD in the Times Beach sample and ~ 800ppb 2,3,7,8-TCDD in the Minker site sample. Mean body weights of experimental groups will be used for calculating quantity of soil required for each group. The soil sample for each animal will be mixed with 5ml distilled water for administration.

#### Soil Samples:

Four soil samples collected from 4 areas in Missouri on March 1, 1983 and delivered to NIEHS on March 3, 1983 were stored in Special Handling Laboratory, NIEHS Warehouse. All manipulations of bulk soil samples were carried out in this laboratory. Samples, identified as AK8201, AK8202, AK8204 and AK8205, were spread on stainless steel trays and allowed to air dry for approximately 40 hours. Each sample, with the exception of AK8204, Minker site uncontaminated, was sieved through 1/4" stainless steel mesh screen to remove rocks and vegetable matter. Sample AK8204 was not sufficiently dry at this point for screening or mixing and was put aside for later processing. Each of the three sieved samples were mixed for 20 minutes in a P-K Twin Shell Laboratory Blender. The blended samples were stored in individual 24 quart stainless steel containers with tops in Special Handling Laboratory, NIEHS Warehouse. An aliquot of each blended soil sample was analyzed and results reported by Dr. J.R. Hass in a memorandum to Director, NIEHS dated April 26, 1983.

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Preparation of Soil Samples for Bioavailability Testing: Sample AK8202, Times Beach contaminated soil - analyzed TCDD concentration  $0.6\mu\text{g/lg}$  soil. 100 grams of sample AK8202, Times Beach contaminated, will be passed through a 60 mesh stainless steel standard testing sieve, collecting fines. Particles not passing through will be weighed and put into glass containers for subsequent disposal by Health and Safety Office personnel. Fines will be collected and put into glass vials with teflon-lined caps in quantity required for each guinea pig in the three groups (no. 4, 5, and 6) receiving this sample. Quantity required will be calculated using mean body weight of each group. An aliquot of fines will be submitted to Analytical Chemistry Group for analyses. Remaining fines will be stored in a solvent cleaned glass vial in Special Handling Laboratory, NIEHS.

Sample AK8205, Minker site contaminated soil - analyzed TCDD concentration,  $0.8\mu\text{g/lg}$  soil. Eighty fine grams of sample AK8205 will be processed in same manner as sample AK8202 and administered to Groups 7, 8 and 9 in quantities required.

Sample AK8201, Times Beach uncontaminated soil. One hundred fifty grams of this sample will be processed in same manner as samples AK8202 and AK8205. Fines will be placed in glass vials in quantities sufficient to equal that given guinea pigs in Group 6. A 30g sample of fines will be spiked with 2,3,7,8-TCDD at a level of  $0.8\mu\text{g}$  TCDD/gram soil by Dr. P. Albro and appropriate amounts of this sample will be given to each animal in Group 11. An aliquot of this sample will be submitted for analyses to Analytical Chemistry Group.

The clay, sand and loam samples will be sieved and spiked in the same manner as sample AK8201. Each animal in Groups 12, 13 and 14 will receive appropriate spiked sample in amounts calculated on mean body weights for each group.

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