UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

**MEMORANDUM**

SUBJECT: Use of historical data in determining the weight of evidence from kidney tumor incidence in the Glyphosate two-year feeding study; and some remarks on false positives

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**BACKGROUND**

The Glyphosate feeding study (EPA Reg. #: 524-308, Caswell #: 661A, Accession #: 251007-014) on Charles River CD-1 mice generated renal tubular adenomas in male mice at the 5000 and 30000 ppm dose levels. The registrant (Monsanto) claims that such tumors are "unrelated to treatment." (ref.1). In support of that they provide historical data from Bio/dynamics and two other laboratories (ref.2).

With respect to historical data we note the large number and variety of factors which influence the life history of rodents in chronic studies. Hence, it is generally agreed that the most relevant historical controls are experiments from the subject laboratory studied within a 3 to 4 year "window" (ref.3).

**SUMMARY**

The main purpose of this memo is to show one way historical data may be used to evaluate the significance of tumors in the glyphosate feeding study. When these data are so used we can conclude that Glyphosate dosing has a statistically significant effect (at the p = .006 level) in the production of kidney tumors in male mice. The appropriate procedure is outlined in the next section entitled Use of Historical Data. The last Section, Remarks on False Positives, addresses some comments by Monsanto (Ref.1) on this subject. That section outlines some of the weaknesses in Monsanto's position.

USE OF HISTORICAL DATA

The following information was derived from Reference 2.

| Data Source* | p (est.of tumor rate) | Sigma (est.of standard deviation) |
|---|---|---|
| Bio/dynamics | .00368 | .00212 |
| IRD Corp. | .00437 | .00109 |
| Combined | .00399 | .00094 |

The value p = .00368, derived from Bio/dynamics data is a reasonable choice to use as a historical control. The data are from the same laboratory that performed the Glyphosate study and are within the appropriate 3-4 year time "window" (ref.3). Further, the standard deviation of the estimate is reasonably small.

We will now examine the Monsanto contention that the kidney tumors are unrelated to treatment. (i.e. Glyphosate has no effect on kidney tumors). First, consider the tumor rate in the Glyphosate Study: 4/198 = .0202 ---

In contrast, Bio/dynamics has the lower historical rate:

3/815 = .00368

The relevant question is: What is the probability that the 198 CD-1 mice in the Glyphosate study will produce by pure chance 4 or more mice with kidney tumors? Another way of stating this is - How likely are we to have a tumor rate of .0202 --- for the Glyphosate study given that the historical rata is .00368?

Questions of this type may be answered from manipulation of the relevant distribution which, in this case is the Binomial:

$$P(r \text{ out of } n \text{ mice have tumors}) = \binom{n}{r} p^r q^{n-r}$$

Where: n = the # of male mice in the study

r = the # of male mice with kidney tumors

p = .00368, the historical probability that an individual male mouse will develop kidney tumors.

q = 1 - p

*This does not include Hazleton Laboratories America, Inc. due to the small sample size of that data set

Using the above distribution and elementary but tedious calculations, we generate the following table:

| # of mice with tumor | Probability that r or more mice will have tumors in a study with 198 male mice |
|---|---|
| r = 0 | 1. |
| 1 | .518177 |
| 2 | .165711 |
| 3 | .037443 |
| 4 | .006481 |

This last table indicates that based on a historical rate of p= .00368 that the probability of seeing 3 or more mice with kidney tumors is about .037; and the probability of seeing 4 or more such mice (i.e. seeing what in fact happened) is about .0064. We note that even considering data from I.R.D., the p value is about .01.

Under such circumstances a prudent person would reject the Monsanto assumption that Glyphosate dosing has no effect on kidney tumor production. Another way of saying this is that if Glyphosate were truly unrelated to kidney production we would expect to see 4 or more tumors in less than 1 out of 100 experiments of the type sponsored by Monsanto. Thus, Glyphosate is suspect.

## REMARKS ON FALSE POSITIVES

In ref. 1 Monsanto notes that "...if 20 types of lesions were evaluated at a probability level of .05, the number expected to be positive would not be one in 20, but rather the probability would be 64 in 100, an unacceptably high value..." Monsanto is referring to the well-known fact that by examining enough data it is likely that one will find an excess of some tumor type by chance alone; thus generating a false positive.

The Monsanto argument required the following assumptions:

1. A mouse may develop 20 distinct and independent (in the statistical sense) types of tumors.
2. The probability of each tumor type in a typical mouse is .05.

It follows from the above that:

$$P(\text{a mouse has at least one tumor}) = 1 - .95^{20} = .6415$$

Hence in 100 mice one would on the average see 64 with tumors. Monsanto proposes to avoid this "problem" of false positives by analyzing the study" ...at the .01 probability level."

We disagree with the Registrants position. First, even if one did analyze the study at the .01 level as they suggest it would still result (using the same mathematics as before) in seeing 18 mice out of 100 with tumors. And hence one still has the problem of false positives from the registrant's viewpoint. But this causes something worse from a regulatory viewpoint. We have decreased the false positive rate (i.e., the probability of saying that a chemical causes tumors when in fact it does not) at the cost of increasing the false negative rate (i.e., the probability of saying that a chemical doesn't cause tumors when in fact it does). The Registrant wishes to avoid false positives while those concerned with the public health wish to avoid false negatives. Hence, for this reason alone Monsanto's argument is unacceptable.

We further disagree as follows:

1. The two assumptions needed to support the Monsanto argument are themselves in need of support (especially the requirement for statistical independence).
2. False positive results are less likely to occur with rare tumors (ref. 5). And the tumors in question are rare.

Viewpoint is a key issue. Our viewpoint is one of protecting the public health when we see suspicious data. it is not our job to protect registrants from false positives. We sympathyze with the Registrants problem; but they will have to demonstrate that this positive result is false.

Finally, we mention that none of the tumors occurred in the control or low dose groups. Instead there was one at 5000 ppm and 3 at the 30000 ppm dose level. This together with the previous comments make it likely that there is a dose-tumor relationship for Glyphosate.

REFERENCES


1. Letter from Monsanto (signed by Frank. S. Serdy) to EPA (Attn: Robert J. Taylor) dated Feb. 5, 1985.

2. Letter from Monsanto (signed by Robert W. Street) to EPA (Attn: Robert J. Taylor) dated March 20, 1984.

3. J.K. Haseman, et al: Use of Historical Control Data in Carcinogenicity Studies in Rodents - Toxicologic Pathology - 12:126-134. 1984.

4. TOX Branch Memo from William Dykstra to Robert Taylor dated 9/4/84.

5. T.R. Fears et al: False-Positive and False-Negative Rates for Carcinogenicity. Cancer Research. 271:1941-1945. July 1977.

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DRAFT

ACCEPTABLE DAILY INTAKE DATA

| RAT,Older NOEL mg/kg | NOEL ppm | S.F. | ADI mg/kg/day | MPI mg/day(60kg) |
|---|---|---|---|---|
| 10.000 | 200.00 | 100 | 0.1000 | 6.0000 |

Published Tolerances

| CROP | Tolerance | Food Factor | mg/day(1.5kg) |
|---|---|---|---|
| Grain Crops( 64) | 0.100 | 13.79 | 0.02069 |
| Avocados( 6) | 0.200 | 0.03 | 0.00009 |
| Citrus Fruits( 33) | 0.200 | 3.81 | 0.01144 |
| Coffee( 36) | 1.000 | 0.75 | 0.01119 |
| Grapes, inc raisins( 56) | 0.100 | 0.49 | 0.00074 |
| Leafy Vegetables( 80) | 0.200 | 2.76 | 0.00828 |
| Nuts(101) | 0.200 | 0.10 | 0.00031 |
| Pome Fruits(126) | 0.200 | 2.79 | 0.00837 |
| Root Crop Veg(138) | 0.100 | 11.00 | 0.03299 |
| Seed&Pod Veg(143) | 0.200 | 3.36 | 0.01098 |
| Palm Oil(202) | 0.100 | 0.03 | 0.00005 |
| Pistachio nuts(210) | 0.200 | 0.03 | 0.00009 |
| Asparagus( 5) | 0.200 | 0.14 | 0.00043 |
| Bananas( 7) | 0.200 | 1.42 | 0.00426 |
| Olives(104) | 0.100 | 0.06 | 0.00009 |
| Stone Fruits(151) | 0.200 | 1.25 | 0.00374 |
| Sugar,cane&beet(154) | 2.000 | 3.64 | 0.10915 |
| Molasses( 96) | 20.000 | 0.03 | 0.00920 |
| Cranberries( 44) | 0.200 | 0.03 | 0.00009 |
| Cottonseed (oil)( 41) | 15.000 | 0.15 | 0.03375 |
| Kidney(203) | 0.500 | 0.03 | 0.00023 |
| Liver(211) | 0.500 | 0.03 | 0.00023 |
| Peanuts(115) | 0.100 | 0.36 | 0.00054 |
| Guava(184) | 0.200 | 0.03 | 0.00009 |
| Papayas(109) | 0.200 | 0.03 | 0.00009 |
| Mangoes( 83) | 0.200 | 0.03 | 0.00009 |
| Soybeans (oil)(148) | 6.000 | 0.92 | 0.08263 |
| Pineapple(123) | 0.100 | 0.30 | 0.00044 |
| Fish,shellfish( 59) | 0.250 | 1.08 | 0.00406 |
| Cucurbits( 49) | 0.100 | 2.84 | 0.00426 |
| Fruiting Vegetables( 60) | 0.100 | 2.99 | 0.00449 |
| Small Fruit,berries(146) | 0.100 | 0.83 | 0.00124 |
| Hops( 73) | 0.100 | 0.03 | 0.00005 |
| Potable Water(198) | 0.500 | 133.33 | 1.00000 |
| Tea(162) | 4.000 | 0.07 | 0.00429 |

| MPI | TMRC | % ADI |
|---|---|---|
| 6.0000 mg/day(60kg) | 1.3686 mg/day(1.5kg) | 22.81 |

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Unpublished,Tox Approved 2F2680,2G2686

| CROP | Tolerance | Food Factor | mg/day(1.5kg) |
|---|---|---|---|
| Soybeans (oil)(148) | 4.000 | 0.92 | 0.05509 |
| Coconut( 35) | 0.100 | 0.03 | 0.00005 |

| | | |
|---|---|---|
| [illegible] | TMRC | %cDI |
| 3.0000 mg/day(60kg) | 1.4238 mg/day(1.5kg) | 23.73 |

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Current Action [illegible]

| CROP | Tolerance | Food Factor | mg/day(1.5kg) |
|---|---|---|---|
| Fish,Shellfish( 5%) | 0.000 | 1.00 | 0.0000 |

| ADI | TMRC | % ADI |
|---|---|---|
| 3.0000 mg/day(60kg) | 1.4238 mg/day(1.5kg) | 23.73 |

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