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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

May 20, 1983 MAY 24 1983

TO: Jay Ellenberger
Registration Division (TS-767)

SUBJECT: Statistical Evaluation of 1980 Acetamide Study and its Relevance to Cancer Risks from Larvin on Cotton and Soybeans ID #0F2413 Action Code: 232

The subject study "Carcinogenesis Bioassay of Acetamide, Hexanamide, Adipamide, Urea and P-Tolylurea in Mice and Rats" by V.R. Fleishman, et. al. (Jour. of Env. Path. & Tox. 3:149-170, 1980) is a poorly designed study which is further marred by gross misuse of statistics and distorted interpretation of the findings. Any use of this study except as an example of what not to do is not recommended.

I. With Respect to design feature:

a. Rat study has 8 compound-dosages groups per sex and mouse study includes 10 such groups. Each group started 50 animals. Thus individual pair-wise comparisons of a compound-dose level with its control (7/sex for rats and 9/sex for mouse) lack power.

b. Acetamide includes one dose-level for rats 2.36% of the diet and mice includes 2 dose levels 1.18 and 2.36%. A dose very close to the original 2.5% (or 25,000 ppm) used in earlier studies which cannot approximate or model physiological responses in study animals or in man.

c. Feeding was only for the first 365 days of study after that time the ground meal mixture of Wayne Lab Blox and study chemical was replaced by Wayne Blox pellets for the last 4 months.

d. Wayne Lab Blox was the study diet used although prior study had pointed up the need to study the effect of arginine-glucuronate on this chemical--for example it is known that acetamide is excreted as a glucuronide. This leads to the expectation that very high dosage levels of test compond could oversaturate the ability of the rat liver to excrete the compound in bound form.

e. Dose, animal age contractor and supplier are confounded in many instances. This does not happen to pertain to the rat acetamide control groups; but it does cast doubt on the data for the low-dose mouse groups.

f. The caging of 5 rats and 10 mice per cage could bias the findings due to crowding.

g. "The number of tissues examined varied between study groups."

II. <u>With respect to Presentation and Analysis of Data</u>:

a. The only data displayed are the total number of animals per group with the tumor of interest and the number examined for the tissue.

b. Data describing weight gain, survival, intercurrent disease, etc. are not even graphically displayed.

c. Selected test results are shown as significant at $p < .05$ or $p < .01$ or not mentioned at all i.e., results of comparisons are not given so that the association can be evaluated for example:

"Clinical Observations

ACETAMIDE Weight curves essentially paralleled each other, and no change in excess of 5% with respect to diet controls was seen in rats or male mice throughout the study. At termination of the experiment, female mice exhibited a weight change not related to dose with 9% depression seen in the low dose and 9% gain in the high dose. No compound related effect was noted on the survival of female rats and male and female mice treated with acetamide. Male rats treated with this compound showed 56% survival at terminal necropsy relative to 86% for diet controls."

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Note that no weight gain or survival curves are shown and that we have not a clue what the weight effect in female mice means because the low dose females come from a different lot and supplier than the high dose mice and the control includes animals from 3 data groups and 2 suppliers (some animals beginning at 42 days of age and others at 58 days. More importantly the survival in male rats is 43/50 controls and 28/50 Acetamide survivors at final kill; this comparison has an adjusted chi-square value of 9.52 which is associated with P <.01. This indicates that there is a apparent excess mortality in Acetamide tested animals,therefore, any evaluation of tumorogenicity should have explored the effect of this factor in the study rats.

If there is a feeling that these liver tumors 0/50 in controls and 41 carcinomas + 1 neoplastic nodules in acetamide fed males and 0/49 control female vs 33 carcinomas and 3 neoplastic nodules in acetamide females are of such importance that they over ride the conditions of experiment. The additional risk estimates can be estimated using Crump's multi-stage model (Global 82) as shown in Table 1.

Note that the data in mice show no statistically significant increase for the mice fed acetamide:

Males		Females
2/91	Control	1/89
0/50	1.18%	2/41
2/46	2.36%	0/46

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Moreover the quantitative risk assessment indicates a 3-30 fold increase, Table 1, of the risk estimates referred to in the Litt memo to O.E. Paynter, February 8, 1983. Note that the estimates for individual sex by study groups show about a 30 fold difference while the combined risks for males and females in the 1980 study are about 3 times larger than the geometric mean of the two earlier studies.

Given the size of the difference in magnitudy there seems to be little benefit in utilizing the newer data (1980), particularly in view of its poor quality. We Recommend that the increased power obtained by using the geometric mean of the two earlier studies supports use of that set of estimates. The CAG water quality document also suggests the use of this approach.

Bertram Litt, Statistician
Toxicology Branch
Hazard Evaluation Division

cc:
ABarton
FBetz
CChaisson
SGross

TABLE I

Potential Carcinogenic Risks to Man from Larvin
based on Rat Bioassays of the metabolite - Acetamide

(mg/kg/d)	Dietary Components	1980 Acetamide Rat Study		Earlier Data: Rat Studies		
		Males	Males & Females	D.J. #2	W.et al	Geometric Mean
1.5×10^{-5}	Cotton-seed oil	1.96×10^{-6}	1.34×10^{-7}	3.25×10^{-8}	6.52×10^{-8}	4.61×10^{-8}
2.3×10^{-5}	Soybean oil	2.97×10^{-6}	2.06×10^{-7}	4.99×10^{-8}	1.00×10^{-7}	7.06×10^{-8}
3.8×10^{-5}	TMRC	4.9×10^{-6}	3.4×10^{-7}	8.25×10^{-8}	1.65×10^{-7}	1.17×10^{-7}
1.25×10^{-5}	Corn, grain	1.6×10^{-6}	1.12×10^{-7}	2.71×10^{-8}	5.44×10^{-8}	3.84×10^{-8}
5.05×10^{-5}	New TMRC	6.5×10^{-6}	4.57×10^{-7}	1.10×10^{-7}	2.20×10^{-7}	1.55×10^{-7}
	Q^*_1	1.291×10^{-2}	8.96×10^{-3}	2.17×10^{-3}	4.35×10^{-3}	3.07×10^{-3}

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

February 8, 1983

TO: Orville E. Paynter, Ph.D.
Chief, Toxicology Branch (TS-769)

SUBJECT: Low-Dose Extrapolation of Carcinogenicity of Larvan Estimated from its metabolite, Acetamide

It has been shown that Larvan metabolizes to Acetamide in the ratio of two mols of acetamide per mol of Larvan. However, because low-dose risks are calculated using exposures measured in mg/kg/day. The two entities may be assumed to have equivalent potencies.

The data utilized below are taken from published articles which report studies of induction and inhibition of liver carcinogenicity in male Wistar rats due to acetamide mixed into ground Wayne Lab Blox. It is important to take note of the design and conduct of these studies which are well described in the publications:

1) Jackson, B. and Dessau, F.I. (1961). Liver tumors in rats fed acetamide. Lab. Invest. 10: 909-923.

2) Weisburger, J.H., Yamamoto, R.S., Glass, R.M. and Frankel, H.H. (1969). Prevention by arginine glutamate of the carcinogenicity of acetamide in rats. Tox. Appl. Pharm. 14: 163-175.

In the Jackson and Dessau paper three experiments are described which include serial kills or changes in dietary supplements which underestimate the effects of 12 month feeding of a fixed dose. However it seems clear that the substudy of 0, 1.2, 2.5 and 5% acetamide shows a time related dose-response which demonsrates a highly statistically significant ($p < .001$)

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linear trend from zero to 2.5% acetamide at the end of 1 year of study when, this relationship is examined including the 5% exposure level we observe a statistically significant ($p < .05$) trend which is comfounded by the non-linearity resulting from the reduced effect in the 5.0% dosage group. Interestingly it was shown in the third substudy that reducing the length of the dosing period increases the liver cancer rate:

	Experiment 1		Experiment 2	
Acetamide Dose	Early Deaths	Total Examined	Early Deaths	Total (12m) Examined
5%	2/11 (18.2)	4/48 (8.33)	0/0	1/18 (5.56)
2 1/2%	----	----	3/4 (75%)	6/22 (27.5)
1.25%	----	----	1/1 (100%)	4/24 (16.7)
Control	0/2	0/43 (0.00)	%	0/25 (0.00)

In the Weisberger et. al. study our primary concern for risk assessment purposes are the findings in the control and 2.5% acetamide groups:

2.5% acetamide	12 mo. liver tumor rats	2/24	Total rats(15 mo.)	12/24
Control	12 mo. liver tumor rats	0/11	Total rats(15 mo.)	0/11

Although not contributing to mathematical extrapolation per se, the biological significance of the almost complete suppression of this effect by the addition of 5.6 arginine glutamate to 2.5% acetamide must be considered separately as part of the weight of the evidence (2/30 animals were diagnosed with hyperplastic nodules or hepatomas by the end of the 15 month study period).

The data from the 3 study groups described above have been fitted by Crump's multistage model to the one-hit model using the reported number of tumor bearing animals and the numbers of animals examined. No corrections or adjustments of the data have been made due to the appearance of dissemenated liver foci by week 15 and the first liver tumor at week 20. The one-hit models produced have been found to conform closely to the actual data as shown by the goodness-of-fit tests written on the

Attachments 1, 2 & 3. the observed slopes (Q(1)) in mg/kg/day are 0.000174, 0.00134 and 0.00307 respectively. However, the sacrifice of one animal per dose per week clearly makes the first Dessau and Jackson result inappropriately low; the one animal per dose sacrifice per month and the lack of high dose effect in the second study makes this study suitable for comparison with the Weisburger study. It seems reasonable to average these last two results by computing the geometric mean of their potency estimators (Q_1^*), (as recommended by CAG Water Quality Document).

$$Q_1^* \text{ D.J. \#2} = 1.62 \times 10^{-8} \div 7.45 \times 10^{-6} = 2.17 \times 10^{-3}$$

$$Q_1^* \text{ W. et al.} = 1.57 \times 10^{-8} \div 3.61 \times 10^{-6} = 4.35 \times 10^{-3}$$

$$\text{Geometric Mean} = \sqrt{(2.17 \times 10^{-3})\ (4.35 \times 10^{-3})} = 3.07 \times 10^{-3}$$

The Geometric mean of the Q_1^* is an estimate of the upper 95% confidence bound on the slope of the dose-response for both experiments and should be used to estimate the lower 95% confidence bound for the virtually safe dose levels associated with hypothetical risk levels.

3.26×10^{-2}	1×10^{-4}
3.26×10^{-3}	1×10^{-5}
3.26×10^{-4}	1×10^{-6}
3.26×10^{-5}	1×10^{-7}
3.26×10^{-6}	1×10^{-8}

However in view of footnote 5 to page 164 of the Weisburger et al. paper:

"Allied Mills, Inc., Chicago, Illinois 60606. In a personal communication, Dr. Jackson (see footnote 2) indicated that for unexplained reasons acetamide was not carcinogenic in Purina Laboratory Chow." I believe that this risk assessment should be ignored as the study findings may very well be an artifact related to an interaction between acetamide and some component of the Wayne Lab blox.

Bertram Litt, Statistician
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Attachment