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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Risk Assessment for Chlorothalonil Based on Diamond Shamrock's Two Year Chronic Mouse Feeding Study. Accession No. 071541.
Caswell No. 215B

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Summary:

The study data analyzed below indicate that chlorothalonil (CTN) is a renal carcinogen in male CD-1 mice. The weight of evidence determination with respect to human carcinogenicity will be made by the Toxicology Branch Cancer Review Committee.

Chlorothalonil has a potency factor Q_1^* of 2.4×10^{-2} for exposure expressed in mg/kg body weight/day.

Background:

The Registrant submitted their own risk assessment. Sufficient methodological detail was not given in their submission to determine precisely why the Diamond Shamrock results were two orders of magnitude lower than that obtained by Crump's multi-stage model (Ref. 1), where this latter model was implemented is accordance to procedures recommended by the EPA draft guidelines.

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Study Description:

The National Cancer Institute Study (NCI-CG-TR-41, 1978) contains evidence that CTN induces renal neoplasm in Osborne-Mendel male and female rats. This prompted Diamond Shamrock Corporation to perform a second study in mice ("a Chronic Dietary Study in Mice with Technical Chlorothalonil," dated April, 1983) to test the null hypothesis that chlorothalonil does not cause kidney tumors. Their two year feeding study used 97.7% CTN, CD-1 mice and was carried out by Bio/Dynamics.

Test mice were assigned randomly to four groups of 60 males and 60 females per treatment. The treatment groups consisted of control, low, medium, and high dose respectively as shown below.

TABLE 1

Experimental Design for the Chlorothalonil Feeding Study

Group	Dose (ppm)	Number of Males	Number of Females
I	0	60	60
II	750	60	60
III	1500	60	60
IV	3000	60	60

The study was initiated February, 1980 and terminated after 24 months. All surviving mice were sacrificed at the end of the study period. Animals dying or sacrificed during the study or at termination were necropsied.

Qualatative Analysis:

The Registrant and D. Ritter, EPA Toxicologist, note average survival in all groups except high dose males; and "food consumption and weight gain were comparable among groups." They both summarize the results by noting that there is nothing in the study which would either cause the tumor data to be excluded or cause difficulties in its interpertation.

Statistical review indicates no discernable strong dose related trends in the mortality of the test animals. However, as noted by the Registrant, mortality is significantly higher for high dose males when compared to controls ($p = .07$ by Fischer's Exact test). Second, female mortality by 18 months was significantly higher than male mortality for corresponding study groups ($p \leq .01$ by Fischer's Exact test). These mortality data are summarized below in Table 2.

TABLE 2

Cummalative Mortality At Six Month Intervals

	MALES				FEMALES			
DOSE (ppm)	6	12	18	24	6	12	18	24
0	1	3	8	29	4	8	20	42
750	0	2	10	35	2	3	18	38
1500	5	7	8	26	3	6	17	37
3000	2	10	13	38	3	9	20	41

Body weights for both male and female for all treatment groups means were comparable to controls for both sexes. Although significant differences were not noted within either sex, the female mice appeared to exhibit greater variability for both within and between group variances.

The tumors of greatest interest were renal tumors in male mice. The data are summarized in Table 3.

TABLE 3

Dose (ppm)	0	750	1500	3000
Response	0/57	6/59	4/59	4/56

Because the tumor rate rises then flattens out by 1500 ppm, it is clear that the departure from linearity explains the lack of a statistically significant dose-response trend ($p = .14$ by the Peto or Armitage-Cochran tests). However, when historical data are utilized (Ref. 2,3) it may be shown that the effect is dose related. This is done by reasoning similar to that given in Ref. 2. Using a background tumor rate of $p = .002$ (estimated from data in Ref. 3), binomial distribution theory implies that the probability of having 14 or more male mice with renal tumors in a group of 231 is less than .0001. Stated more formally, the dose effect of chlorothalonil is statistically significant at the $p = .0001$ level, compared to the referenced historical controls under the binomial distribution assumption.

Quantitative Risk Assessment:

In addition to the renal tumors noted above, all treatment groups (in both sexes) exhibited gastric carcinomas. These are summarized below.

TABLE 4

Gastric
(Number of Tumors/Number of Animals at Risk)

	0 ppm	750 ppm	1500 ppm	3000 ppm
Female				
Squamous cell Carcinoma	0/57	2/60	6/58	5/58
Glandular	0/57	1/60	1/58	2/56
Total	0/57	3/60	7/58	7/58
Male				
Squamous cell Carcinoma	0/55	2/59	5/59	1/51
Glandular	0/55	1/59	2/59	0/51
Total	0/55	3/59	7/59	1/102

Squamous cell and Glandular carcinomas are not normally additive. However, in this case Dr. L. Kasza, Staff Pathologist, suggests that there may be evidence of multiple tissue tumors that may be due to the same causative agent or mechanism.

For risk assessment purposes we will use the rare renal tumors rather than gastric tumors because that effect is detected at a lower dose. The problem of the non monotonicity of the dose response with the renal tumors can be dealt with by eliminating the 1500 and 3000 ppm dose groups as recommended by the Crump multi-stage procedure and the Mantel/Tukey paper (Ref. 6). This approach is consistent with EPA policy (see Ref. 4) that tends to select the data groups giving the highest potency (Q_1*).

Crump's multi-stage procedure was applied to the following renal-tumor-data set where human equivalent dose is expressed in mg/kg/day.

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TABLE 5

Renal Tumors

Human Equivalent Dose (mg/kg/day)	0	8.2
Response	0/57	6/59

The human equivalent dose (in the absence of experimental data) was calculated by standard methods (see Appendix for formulas).

The results of the multi-stage modeling are given below.

MLE of Q_1	Est of Q_1*
1.31×10^{-2}	2.4×10^{-2}

Note that the Chi Square value is not shown, as it is not relevant because there are only two dose groups to fit. Note that the MLE (maximum likelihood estimate) of Q and Q_1* are close. Hence, there is a close correspondence between the point estimate of the slope based on the data, and the 95% upper bound on this slope.

Diamond Shamrock carried out their own independent risk assessment producing results which differ from ours by about two orders of magnitude. This discrepancy might be reconciled as follows:

1. If the Registrant used all four groups without surface area adjustment of the dose and if they used the maximum likelihood estimate for potency (instead of $Q_1^* = 2.4 \times 10^{-2}$), their estimate would be 2.8×10^{-3}.

2. If the Registrant also performed a surface-area correction of say $(6000/40)^{1/3} = 11.4$, they would find a potency, Q_1*, of about 2.45×10^{-4}.

3. By working backwards from the Registrant's risk data we have found that their potency was about 2.28×10^{-4} to 2.46×10^{-4}. This includes the 2.45×10^{-4} value calculated above. That possibly clarifies the two orders of magnitude differences between the results.

For completeness, we list two other possible sources of error:

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1. The Registrant appears to count all animals on test while Toxicology Branch reviewers count only non-autolyzed mice.

2. The Registrant appears to over estimate the "Annualized Daily Exposure" by not taking into consideration that a worker will generally be exposed for only 1/2 his(her) life time.

Characterization of Risk:

The risk for the TMRC and some of the published tolerances (see Appendix for complete list) are given below where the risk are based on a $Q_1^* = 2.4 \times 10^{-2}$.

TABLE 6

	Exposure (mg/kg/day)	Risk
Celery	.001073	10^{-5}
Cucumber	.000907	10^{-5}
Melons	.002504	10^{-5} to 10^{-4}
Beans (snap)	.001226	10^{-5}
Tomatoes	.00359	10^{-4}
Cabbage	.0009198	10^{-5}
TMRC	.011905	10^{-4}

Worker risks were obtained from S.E. Noren's memo to R. Engler dated December 17, 1984 (Ref. 5), the basic data and risks are given below.

TABLE 7

Worker Risks Based on $Q_1^* = 2.4 \times 10^{-2}$ and 100% Dermal Penetration

Ground Application	LADD[a]	Risk[b]
Sprayer Mixer	.0415	10^{-3}
Aerial Application		
Mixer	.029	10^{-4} to 10^{-3}
Flagman	.011	10^{-4}
Pilot	.005	10^{-4}

[a] LADD = Lifetime Average Daily Dose (see Appendix for detail).

[b] Risk = $Q_1^* \times$ LADD

APPENDIX

I. Reference

II. Formulas

III. Published Tolerances

I. REFERENCE

1. Crump, K. S. (1982) An improved procedure for low-dose carcinogenic risk assessment from animal data. Journal of Environmental Pathology and Toxicology Vol. 5, No. 2, 675-684.

2. Memorandum, H. Lacayo to R. Engler. Subject: Use of Historical Data..... dated Feb. 29, 1985.

3. Letter. R. P. Burton of Biotech to H. M. Jacoby of EPA dated Dec. 19, 1983.

4. Water Quality Criteria Documents, Federal Register, Vol. 45. No. 231, Friday, Nov. 28, 1980.

5. Memorandum. S. E. Noren to R. Engler, Subject: Applicator Exposure for Chlorothalonal.

6. N. Mantel, N. R. Bohidar, C. C. Brown, J. L. Ciminera, and J. W. Tukey. An improved Mantel-Bryan procedure for "safety" testing of carcinogens. Cancer Research 345, 865-872 (1975).

II. FORMULAS

A. LADD Formula

The Lifetime Average Daily Dose (mg/kg/day is approximated by:

LADD = (Dose acquired in one working day in mg/kg/day)

x (No. of working days per year with the chemical)/365

x (35 years of working)/(70 years lifetime)

$$= (\text{One day exposure}) \times \frac{\text{days exposed/yr}}{365} \times \frac{35}{70}$$

B. Conversion of ppm to mg/kg/day

1 ppm in mouse diet = .150 mg/kg/day

Quick Conversion (for ppm only)

$$1 \text{ ppm in diet for animal} = \frac{\text{(Wt of diet in grams)}}{\text{(Wt of animal in grams)}}$$

= mg/kg/day for animal

C. Interspecies Conversion Factor

Let SA = Surface Area

W_h = body weight of humahn
W_a = body weight of animal
d_h = dose for human (mg/kg/day)
d_a = dose for animal (mg/kg/day)

If we assume the surface ares is proportional to $W^{2/3}$ and that equivalent doses (in mg/day) are proporational to surface areas, then $d_h = d_a \times (W_a/W_h)^{1/3}$.

For example extrapolation of mouse to an "equivalent" human dose can be done as follows:

1. Convert mouse dose which is usually in ppm to mg/kg/day.

   .15 x (mouse dose in ppm) = mouse dose in mg/kg/day.

2. Therefore,

   Human Equiv. Dose = (mouse dose in mg/kg/day)$\times(25/65000)^{1/3}$