3-23-83 Caswell #1

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

3-23-83

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Dimethoate R.S./3(c)(2)(B) : Review of Mutagenicity Studies

TO: Amy S. Rispin, Ph. D.,, SIS
Hazard Evaluation Division (TS-769)

In re: Rodent dominant lethal tests, and cytogenetic analyses.

Attached are evaluations (DER's) on two mutagenicity studies submitted by American Cyanamid (Accession No. 249601) in response to the Dimethoate Registration Standard data call-in.

Both studies have been judged UNACCEPTABLE according to recognized standards of testing and criteria employed for such assays in the Toxicology Branch.

3-23-83

Irving Mauer,,.Ph. D., Geneticist
Toxicology Branch, HAZARD EVALUATION DIVISION (TS_769)

DIMETHOATE

Caswell 358

(DER) DATA EVALUATION RECORD

TEST ARTICLE: [redacted] stated as "99% pure," and dissolved in saline for assay.

CITATION: "Genotoxicity of organophosphorus insecticide Dimethoate in mouse," (undated), by N. Degraeve and J. Moutschen (Universite de Liege, Laboratoire de Genetique, 15, rue Forgeur, B-4000 Liege, Belgium.; submitted by American Cyanamid Co. on behalf of the Dimethoate Task Force. [Exhibit C-II, Accession No. 249601.]

TRADE SECRET CLAIM: CBI

REASON FOR REVIEW: Response to Dimethoate RPAR/3(c)(2)(B)

REVIEWED BY: Irving Mauer, Ph.D. HED/TB

APPROVED BY: (DATE)

DATE OF REVIEW: March 22, 1983 3/24/83

TEST TYPE: Rodent cytogenetics: (1) Dominant lethal test (DLT); (2) clastogenicity analysis in bone marrow and spermatogonia (CA).

STUDY - 1: Male mice of the Q (Edinburgh) strain (maintained by D. S. Falconer, Institute of Animal Genetics) were given test article according to two dosage schedules: (i) 10 mg/kg, ip, once (5 males); or (ii) 0.6 ppm in drinking water 5 days a week for 7 weeks (20 males), and then each mated with 4 untreated females per week for 7 weeks (acute test), or at the end of treatment (chronic test). Pregnant females (ascertained by vaginal plug) were sacrificed on day 14 of gestation, and the following reproductive parameters enumerated: corpora lutea, deciduomata (visible post-implantation losses), and pre-implantation losses (calculated). Both negative (20 males given saline) and positive (the mutagen, methylmethanesulfonate, MMS, at 60 mg/kg, ip, once) controls were included in the assay. No deaths and "reduced toxicity" were reported on these schedules.

Tabulated data recorded no induced dominant lethality (post-implant or pre-implant losses) in mice treated with test article by either schedule or route of administration (Tables I and II), contrasted with the expected response for MMS-treatment (positive control).

STUDY - 2: The same dosage schedules and routes were used for cytogenetic analysis. [NB: It is not clear in the report, however, whether different groups of mice were used for this assay.] Males were sacrificed 12, 24, 36 and 48 hours after acute ip treatment (2 animals per sampling time), or at the end of the chronic oral treatment (3 animals). Chromosomes were examined in at least 100 Giemsa-stained bone marrow and spermatogonial metaphases per animal, reported in two tabulations. MMS (60 mg/kg, ip, once) also served as positive control for this assay.

Tables III and IV record no increase in structural chromosomal damage (fragments, exchanges, or gaps) in dimethoate-treated animals, compared to the expected response to MMS-treatment in both cell preparations.

The authors conclude "...that at the doses used Dimethoate does not show any genotoxicity in mouse either after acute or chronic treatments."

EVALUATION: Both these studies are UNACCEPTABLE as comprehensive assays for dimethoate's cytogenetic potential, due to the following deficiencies:

STUDY - 1 (DLT):

1. No information was presented on the treated males, which are the appropriate experimental units in dominant lethal assays, namely: age, sex, number employed in sequential (acute) tests; fertility, impregnation rates, etc.

2. Only single dosage levels were used, and those presumably insufficient since no clinical effects were (apparently) observed.

3. No statistical analysis was presented.

4 No positive control was employed for chronic test.

5. Insufficient number of pregnant females were sampled.

STUDY - 2 (CA):

1. Insufficient single dosage levels were used for both schedules (no clinical effects observed).

2. Inadequate dosage schedules were employed.

3. No numerical analyses on chromosome number per cell were carried out.

4. The numbers of control animals were not stated. (saline or MMS).

5. No statistical analysis was performed.

Caswell #1

DIMETHOATE

Caswell # 358

(DER) DATA EVALUATION RECORD

TEST ARTICLE: Dimethoate (0,0-dimethyl S-methyl carbamoylmethyl phosphorodithioate), re-crystallized from the technical to yield no detectable impurities (by TLC); dissolved in sterile 0.9% NaCl for assay.

CITATION: EFFECT OF DIMETHOATE AND O-DEMETHYLDIMETHOATE IN THE DOMINANT LETHAL TEST ON MICE (Translation from the German: "Zur Wirkung von Dimethoat and O-Desmethyldimethoat im Dominanten Letaltest an der Maus"), by G.W. Fischer and H. Scheufler. Wiss. Z. - Martin-Luther Univ. Halle-Wittenberg, Math.-Naturwiss. 30:21-29 (1981).

REASON FOR REVIEW: Submitted by American Cyanamid in response to 3(c)(2)(B) [Accession No. 249601].

REVIEWED BY: Irving Mauer Ph.D.
HED/TB

Approved by:
(Date)

DATE OF REVIEW: March 15, 1983

TEST TYPE: Rodent Dominant Lethal Test.

STUDY: Juvenile (12 wk. old) male mice of two inbred strains (AB Jena-Halle; DBA) were injected (ip) with saline solutions of re-crystallized, impurity-free (by TLC detection) dimethoate, or its demethylated derivative (as the sodium salt), and mated for "five weeks with three females of the same strain per day." Three dosage schedules were employed: (i) Single doses of 30 or 60 mg/kg dimethoate, or 35, 69 or 690 mg/kg demethyldimethoate (5 tests in all); (ii) five consecutive daily doses of 6 mg/kg dimethoate (2 tests) or 7 mg/kg dimethydimethoate (3 tests); and (iii) three daily doses of 18 mg/kg dimethoate, or 21 mg/kg dimethydimethoate (1 test each). Pregnant females were autopsied on the 18th day following detection of a vaginal plug, and the following reproductive parameters enumerated: Total weekly numbers of corpora lutea (CL); dead (Tl) and/or live (Ll) implants for experimental (T) and control (K) groups. Induced lethals in experimental (test compound) and control (saline) groups were then compared for each of the five weeks by four-cell chi-square (corrected by Yates procedure for low occupancy of individual cells), using the ratios of group dead-to-total implants (Tl_T/Gl_T and Tl_K/Gl_K, i.e., post-implantation

loss) and group live implants-to-corpora lutea ($1-Ll_T/CL_T$ and $1-Ll_K/CL_K$, i.e., total loss), the quotents partitioned as follows:

$$X^2\ (Tl/Gl) = \frac{[lTI_TLI_K - TI_KLI_TI - 0.5(GI_T + GI_K)]^2\ (GI_T + GI_K)}{GI_TGI_K(TI_T + TI_K)\ (LI_T + LI_K)}$$

and for the comparison of the quotients LI/CL:

$$X^2\ (Ll/CL) = \frac{[lCL_TLI_K - CL_KLI_Tl - 0.5(CL_T + CL_K)]^2\ (CL_T + CL_K)}{CL_TCL_K(LI_T + LI_K)\ (CL_T + CL_K - LI_T - LI_K)}$$

Statistical significance (by rejection of the null hypothesis) was ascertained if $TI_T/GI_T > TI_K/GI_K$ or $LI_T/CL_T < LI_K/CL_K$, and $X^2 > X^2_1\ (2 - 0.005) = 2.7055$ (one-sided test with 5% significance level).

The results were summarized in four tabulations, and reported as:

(i) "...No significant increases in post-implantation loss (TI/GI) or total loss (1-LI/CL) in either strain for either compound at either the lower or higher dose after single i.p. administration (Tables 1 and 2)...." [although the authors noted non-reproducible isolated changes (<5%) in total losses during single weeks for both compounds];

(ii) "...After the i.p. administration of 6 mg/kg dimethoate I or 7 mg/kg demethyldimethate for five consecutive days to male mice of the strain AB Jena-Halle, significant effects of both substances (ranging to <0.005) in the first, but not the second set of tests (Table 3)."

(iii) "...Repeat test in DBA mice given consecutive daily doses over 3 days (at 18 mg/kg/day or 21 mg/kg/day)" ...also did not lead to any significant increase in the post-implantation loss (Test 9, Table 4)."

Highly significance increases were recorded for both parameters with both strains treated with the mutagen (150 or 300 EMS).

The authors concluded that "...dimethoate, in the form of its pure active substance, does not induce any measurable number of dominant lethal mutations in the male germ cells of mice of the strains AB Jena-Halle and DBA after the administration of 30 and 60 mg/kg under the test conditions indicated..." [single or multiple i.p. dose schedules]....[and]... "...The O-demethyl derivative II, which is significantly less toxic than I [dimethoate] and can therefore be tested at higher dosages, does not show any mutagenic effect even after a single dose of 690 mg/kg given to male mice of the DBA strain."

EVALUATION: This (published) study is considered inadequate (i.e. UNACCEPTABLE) as a comprehensive test for dominant lethals in mice, in part due to the following deficiencies which compromise the stated (negative) conclusions: "...we are unable to derive any statistically convincing indication of the development of lethal mutations...":

1. The number of males of each strain treated (and mated) is not stated;

2. Inappropriate use of the females as experimental units; and no fertility data (pregnancy rate, etc);

3. Inappropriate negative control (and diluent) for dimethoate, namely saline;

4. No reported clinical effects of dimethoate dosages used in the males, presumably at the injected LD50 of 45 mg/kg, according to published values; all indicating

5. The potential for insufficient compound transporting to germ cells to be effective.