TOX REV 270 ✓ OK

DATE: July 12, 1978

SUBJECT: Validation review of IBT studies.
Glyphosate Caswell No. 661A

FROM: D. G. Van Ormer, Ph.D.
Toxicology Branch, HED (TS-769) DVO

TO: Mr. Robert Taylor (PM #25)
Registration Division (TS-767)

Description

The following IBT toxicology studies were "validation reviewed" by this reviewer during the period 30 June to 12 July 78. Four of the studies could not be validation reviewed because raw data had not been provided. Several studies, as indicated, required reviewing prior to validation review.

IBT No.	Studies
651-05275	Teratology - See Comment #1
621-05412	4-day fish, N.A. for TOX
601-05848	Acute dermal (mice) - See Comment #2
663-06290	BTL 74-116A, no raw data, acute inhal.
663-06290	BTL 74-116B, no raw data, subacute inhal.
601-06527	Acute ChE inhibition in rats
633-07801	No raw data, Rec-assay
633-07507	No raw data, Ames test
623-07508	See Comment #3 (host-mediated mutagenicity)
8580-09117	Hen neurotoxicity

Of the above listed studies for which raw data had been provided none contain either audit or validation statements which, in my opinion, change the conclusions of any of the EPA reviews of these studies.

In No. 623-07508 the approximately 12% of the reported data are judged by this reviewer as adequately representative of all raw data submitted, including both reported and non-reported data.

COMMENT #1

Review of Teratology Study (rabbit), IBT 651-05275, supporting "Roundup" (Monsanto; EPA Reg. No. 524-308; Caswell No. 661A).

Description of Test Material - Organophosphate herbicide, technical material (glyphosate, CP 67573, N-phosphonomethylglycine).

Recommendation

Repeat the study using at least 3 doses in a range such that there is a substantial difference in maternal toxicity between the high and low doses. In many cases an appropriate range is between 1/16 and 1/2 of the maternal 10-day oral LD-50 dose. Report the age of rabbits, list tissues examined, report the average fetal weight per sex, and tabulate crown-rump measurements.

Review:

Fifty-six albino doe rabbits were divided into four equal groups: control, positive control (Thalidomide, 75 mg/kg), and two test groups receiving, respectively, doses of 10 and 30 mg/kg/day of CP 67573 (technical glyphosate, "Roundup"; oral LD-50, rabbits 3800 mg/kg). The substance was administered during organogenesis (gestation days 6 through 18) via gelatin capsule, with an empty capsule to controls. Rabbits were weighed on days 0, 6, 9, 12, 15, 18 and at sacrifice (day 29). Each animal received luteinizing hormone at artificial insemination, and was housed individually. The rabbits were observed daily. Immediately after caesarian section, viable young were thoroughly examined, weighed and placed in an incubator at 37°C. Observations for viability (respiration and paw movements) were made hourly for 7 hours and again at 24 hours. Careful dissection of the young was followed by examination for abnormalities in size, shape, and orientation of organs and vessels. Skeletal examination included Hurley's alizarin-staining technique for embryo tissue.

Results

Body weights of test groups remained virtually constant during dosing and throughout the study. Positive control weights declined from day 6 to day 29. Untreated controls gained weight slightly during the study. Neither deaths nor abnormal behavior occurred in the does during the investigation. With regard to reproductive effects, the number of live young produced by both treatment groups was 84% of the control value. Although percent resorption was 2.5 times control for the low dose, there

was no effect at the high dose. The ratio of live young to number of implantation sites also showed a change (decrease) at the low dose while no effect appeared at the other dose. Examination for gross external fetal abnormalities revealed no abnormal fetuses in either treatment group (167 fetuses) and one umbilical hernia in the control group (101 fetuses). Progeny body weights (except for positive controls) showed no change from controls. The Viability Index (No. viable at 24 hours/No. viable at birth) was the same as untreated control for both groups. Dissection of young from rabbits treated with CP 67573 revealed no gross internal abnormalities. Inspection of fetal skeletal development exhibited a slight increased incidence of supernumerary ribs compared to controls. However, the incidence of supernumerary ribs in controls was five times lower than for historical controls from the same laboratory.

Classification: Unacceptable

1. Rational for dose choice not stated.*
2. Only two dose levels utilized; low dose and high dose did not produce significantly different maternal effects.

In addition

1. Age of rabbits not reported.
2. Specific list of tissues examined not given.
3. Average fetal weight per sex not reported.
4. Crown-rump measurement not made.

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* Doses too low compared to oral LD-50 rabbits (3800 mg/kg).

COMMENT #2

Review of Acute Subcutaneous Toxicity Study (mice), IBT 601-05848, supporting "Roundup" (Monsanto; EPA Reg. No. 524-308; Caswell #661A).

Description of Test Material - Organophosphate herbicide, "Roundup", formulation MON-2139 QHD-13.

Recommendation

Retain this review in files as Core minimum data.

Review

The young albino mice ARS_2 Sprague-Dawley strain, after health and suitability testing, were injected subcutaneously in the dorsal cervical region. The four dosage groups each contained 5 males and 5 females. Dose levels were 1350, 2025, 3038, and 4556 mg/kg. The material administered was identified as MON-2139, QHD-13 (Roundup) undiluted. The mice were individually housed in wire-mesh cages and observed for 14 days. Initial and final body weights, mortalities, and reactions were recorded. A necropsy examination was conducted on all animals. The LD-50 calculation was after Litchfield and Wilcoxon in so far as possible.

Results

LD_{50} (S.C., mice) = 1720 mg/kg
95% Conf. wt. = 53%
Toxic Signs: Labored breathing, ptosis, tremors, diuresis, hypothermia, muscular weakness, prostration.

Necropsy: fluid and necrotic tissue at the injection site, which had exhibited severe edema. No other gross pathological reactions were noted except slight enteritis.

Classification: Core minimum data. Although substance identification in the actual study was by code number only we regard Monsanto's submission of this study in the Glyphosate package as sufficient evidence for compound identification.

COMMENT #3

Review of Host-Mediated Assay for Detection of Mutation Induced by CP 67573 (Test Species: Albino Rats and Mice), IBT No. 623-07508, supporting "Roundup" (Monsanto; EPA Reg. No. 524-308; Caswell #661A).

Description of Test Material - Organophosphate herbicide, technical material (CP 67573, glyphosate, N-phosphonomethylglycine).

Conclusion:

This study presents adequate evidence for lack of mutagenicity in the described rat- and mouse- mediated bacterial reverse mutation tests.

Recommendation:

This study is acceptable according to recent EPA Guidelines for mutagenicity testing.

Review

The test material (CP 67573; Roundup, Batch XHB-87, 75%) was examined *in vivo* for reverse mutation by host-mediated assay utilizing albino rats, albino mice, and *S. typhimurium*. Four animals of each species were gavaged on five consecutive days at doses of either 100 or 300 mg/kg. On the fifth day, appropriate volumes of the histidine-dependent Salmonella culture (his G 46; incubated overnight in sterile nutrient broth) were injected into the peritoneal cavity of each animal immediately after the last gavage. Positive controls received an intramuscular injection of 100 mg DMN/kg (for rats) or 30 mg MMNG/kg (for mice) at the time of bacterial injection.

Following CO_2 -asphyxiation, three hours after inoculation, each carcass received 1 ml (i.p.) of sterile, normal saline. Appropriate aliquots of diluted peritoneal fluid (aspirate) were plated on sterile agar, either with histidine (for total plate counts) or without histidine (for reverse mutant counts). Incubation was at 37°C for 48 hours.

Results

Neither deaths nor untoward behavior reactions occurred. Mutation rate was calculated as revertants per ml of aspirate on deficient agar divided by total organisms per ml of aspirate on complete agar. In the rat-mediated trial No. 2 the average mutation rate for the low dose of test chemical was 74% of the positive control (dimethylnitrosamine administered at 1/5 the total test dose). In addition, the average mutation rate at this

dose was six times the untreat-control rate. However, since two of the three total-organism plate counts at this dose are several (2- to -8) fold lower than any of the rest of the 24 plate-count averages for the whole study, this trial (No. 2) should be questioned. Trial No. 1 does not demonstrate increased mutational rate of treated organisms over controls. When activated in mice, the mutation rates of both test doses were similar and less than untreated controls, while the positive controls (MNNG) averaged 32 times untreated controls.

Classification: Adequate study.

1. Protocol source not referenced
2. Number of animals at each dose is too small considering the unsatisfactory precision of some trials.
3. Data for all animals should be presented, whether included in averages or not.
4. Appeared to be too many counts on some plates for adequate counting.

Additional notations:

1. Composition of media, quantitative statement of dilutions, etc. not stated.
2. Since only one dose was utilized for positive control, sensitivity of the system to show dose-effect relation is not demonstrated for present conditions.
3. Similarity of activation requirements for test material and positive control not discussed.

D L Van Orner
18 Aug 78
E 8/23/78