Glyphosate/Tox 6-17-80 (1)

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

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releasable

MEMORANDUM

OFFICE OF TOXIC SUBSTANCES

SUBJECT: EPA Reg.#524-308, Glyphosate; Submission of rat teratology, rabbit teratology, dominant lethal mutagenicity assay in mice.
CASWELL#661A Accession#242516

FROM: William Dykstra
Toxicology Branch, HED (TS-769)

6/17/80

TO: Robert Taylor (25)
Registration Division (TS-769)

Recommendations:

1) With respect to the rat teratology study, the registrant is requested to explain the multiple malformations in the fetuses, in addition to bent tail, of dam 20091. While bent tail has a historical incidence of 5 fetuses (1 litter) out of 524 litters examined, the remaining multiple malformations in the fetuses of that litter (20091) may not be explained by the historical data presented. A repeat of the study could confirm whether the findings are compound related.

2) With respect to the rabbit teratology study, the registrant is requested to explain the multiple malformations of one fetus in litter 2284 (high dose), whereas no multiple malformations are noted in the historical data. In addition the incidence of scoliosis and malformed ribs is greater in the test groups than historical controls. A repeat of the study could confirm whether the findings are compound related.

3) The dominant lethal assay in mice study is acceptable as core minimum data.

Review:

1. Teratology Study in Rats (IRDC Report No. 401-054; March 21, 1980)

Test Material: technical glyphosate; 98.7% assay; Lot XHJ-64; white powder.

One hundred untreated sexually mature virgin female Charles River COBS CD rats (The Charles River Breeding Labs, Inc., Portage, Michigan) were used in this study to determine the teratogenic potential of technical glyphosate. The rats were approximately 14 weeks old when mated and had been acclimated in the laboratory for a minimum of 10 days prior to study initiation. Each rat was assigned a unique number and ear tagged for identification when placed on study. All rats were individually housed, except during mating, in suspended wire-mesh cages and maintained in a temperature-, humidity- and light-controlled environment. Purina Rodent Laboratory Chow 5001 and tap water were available ad libitum.

Mating was initiated on April 16, 1979 and the last cesarean section was performed on May 12, 1979. One female and one female rat of the same strain were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug or by a vaginal inspection for sperm. The day that mating was detected was designated day 0 of gestation and the female was returned to an individual cage. Mated females were consecutively assigned in a block design to a control group and three treatment groups consisting of 25 rats each. The test material was prepared in 0.5% aqueous Methocel to permit administration at dosage levels of 300, 1000, and 3500 mg/kg/day at a constant volume of 10 ml/kg. The test material was administered orally by gavage as a single daily dose on days 6 through 19 of gestation. The control group received the vehicle only on a comparable regimen at a volume of 10 ml/kg. Individual dosages were determined from individual body weights recorded on gestation day 6.

Prior to treatment, the females were observed daily for mortality and overt changes in appearance and behavior. The females were observed daily for mortality and clinical signs of toxicity on days 6 through 20 of gestation. Dams not surviving to the scheduled sacrifice were necropsied in an attempt to determine the cause of death. Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20.

On gestation day 20, all surviving females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the uterus was excised and weighed and the fetuses removed. The location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded.

All fetuses were individually weighed and examined for external malformations and variations, including the palate and the eyes. Each fetus was externally sexed and individually numbered and tagged for identification. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning as described by Wilson. The remaining one-half of the fetuses were fixed in alcohol, macerated in potassium hydroxide, and stained with Alization Red S by a method similar to that described by Dawson for subsequent skeletal examination.

Statistical Analysis of the data was performed.

Results:

There were no biologically meaningful differences in appearance or behavior attributable to treatment with technical glyphosate in the 300 or 1000 mg/kg/day dosage groups when compared to the control group. Soft stool, diarrhea, or both were noted at least once during the treatment period (in all but three rats) in the 3500 mg/kg/day dosage group, with diarrhea occurring primarily either prior to death or during the last days of treatment. Breathing rattles and inactivity were noted only in rats in the 3500 mg/kg/day dosage group and red matter in the region of the nose, mouth, forelimbs or dorsal head was noted prior to death. Six rats in this dosage group died, one each on gestation days 10 and 17 and two each on gestation days 11 and 12. Stomach hemorrhages were noted in two of these rats at necropsy, however, a cause of death could not be determined for these six dams. One incidence of splenic necrosis and adhensive peritonitis was found in the 300 mg/kg/day dosage group at necropsy examination.

There were no biologically meaningful differences in mean maternal body weight gain in the 300 and 1000 mg/kg/day dosage groups when compared to the control group. However, a definite reduced mean maternal body weight gain was noted in the 3500 mg/kg/day dosage group over the treatment period due to mean maternal body weight loss during the first three days of treatment. There were no biologically meaningful differences in the mean number of viable fetuses, late or early resorptions, postimplantation loss, corpora lutea, the fetal sex distribution or mean fetal body weight in the 300 and 1000 mg/kg/day dosage groups when compared to the control group and nonviable fetuses were not present in any group.

In the 3500 mg/kg/day dosage group, there were no late resorptions although a statistically significant increase in the mean number of early resorptions resulted in a slight increase in mean postimplantation loss. A statistically significant decrease in the mean number of total implantations, viable fetuses, and mean fetal body weight and a slight decrease in the mean number of corpora lutea was noted in this group. No statistically significant difference in fetal sex distribution occurred in the high dose group.

There were no malformations in the 300 and 1000 mg/kg/day dosage groups. In the 3500 mg/kg/day dosage group, the number of litters (3) with malformations was identical to the control group. However, several fetuses with the anomally classified as dwarfish or bent tails were found in single litters. Also, the fetuses had multiple malformation in addition to bent tails. As a result, an increae in the number of fetuses with malformations was noted in this group when compared to the control group. These increases were considered genetic in origin as bent tail and dwarfism occurred in several fetuses in a single litter in the historical control data.

Developmental and genetic variations in the 300 and 1000 mg/kg/day dosage groups were comparable to the control group. An increase in the number of litters and fetuses with unossified sternebrae was noted in the 3500 mg/kg/day dosage group and was considered a developmental and was considered a developmental variation.

Conclusion: It is uncertain whether technical glyphosate was not teratogenic at dosage of 3500 mg/kg/day during days 6 to 19 of gestation. The NOEL for maternal and fetal toxicity is 1000 mg/kg/day.

Classification: Core-Minimum Data

2. Teratology Study in Rabbit (IRDC Report#401-056, Feb. 29, 1980)

Test Material: technical glyphosate; 98.7% assay; Lot#XHJ-64

Groups of 16 pregnant Dutch Belted rabbits were used to determine the teratogenic potential of technical glyphosate. Dosage levels of 0, 75, 175 and 350 mg/kg/day were administered orally by gavage as a single daily dose on days 6 through 27 of gestation at a constant volume of 1 ml/kg. The control group received the vehicle only, 0.5% aqueous Methocel, on a comparable regimen. Prior to treatment, the females were observed daily for mortality and overt changes in appearance and behavior. The females were observed daily for mortality and clinical signs of toxicity on days 6 through 28 of gestation. Dams not surviving to the scheduled sacrifice were necropsied in an attempt to determine the cause of death.

Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24, and 28. On gestation day 28, all surviving females were sacrificed by injection of an overdose of sodium pentobarbital into the marginal ear vein.

Immediately following sacrifice, the uterus was excised and weighed and the fetuses were removed. The number and location of viable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident mortphological changes and the carcasses discarded.

All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was dissected, internally examined for visceral malformations and variations, including the brain by a mid-coronal slice. The heart was dissected by a modification of the method described by R.E. Staples. The eviscerated, skinned fetuses were individually numbered and tagged for identification, fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson for subsequent Skeletal examination. Statistical Analysis of the data was performed.

Results:

No treatment-related abnormal clinical signs were observed in rabbits dosed at 75 mg/kg/day. A slight increase in the incidence of soft stools and diarrhea was noted in the 175 mg/kg/day group and a definite increase in these signs and nasal discharge were noted in the 350 mg/kg/day group compared to the control group. The mean maternal body weight gain for each treated group was comparable to that of the control group. One rabbit in the 75 mg/kg/day dosage group died apparently of pneumonia. Two rabbits in the 175 mg/kg/day group and ten rabbtis in the 350 mg/kg/day group died during the study. The cause of death for most of the these rabbits could not be determined at necropsy. Two rabbits in the control group, one in the 175 mg/kg/day group aborted and were sacrificed.

There were no biologically meaningful differences in mean number of viable fetuses, early or late resorptions total implantations, corpora lutea, or fetal sex distriutions. A slight decrease was noted for mean fetal body weight of all of the treated groups compared to the control group. However, mean fetal body weights for all groups are comparable to historical control mean fetal body weight values.

The number of litters with malformation in any of the treated groups was not significantly different from the controls. A few malformations were noted in each of the three treated groups. Since these did not occur in a dose-related pattern and their frequency was comparable to that of the historical control group, they are not considered treatment-related. The number of fetuses and litters with developmental and genetic variations were comparable for all groups.

Conclusion: It is uncertain whether technical glyphosate was not teratogenic at 350 mg/kg/day or less. Glyphosate was maternally toxic at 350 mg/kg/day. The NOEL for maternal toxicity is 175 mg/kg/day.

Classification: Core-Minimum Data

3. Dominant Lethal Mutagenicity Assay with technical glyphosate in Mice (IRDC Report#401-064; April 16, 1980)

Test Material: technical glyphosate; 98.5% assay; Lot#XHJ-64.

Glyphosate was administered once by gavage on the first day of the study to groups of 10 male Charles River CD-1 mice at dosage levels of 0, 200, 800, and 2000 mg/kg body weight. Glyphosate was administered as a suspension in aqueous 0.5% Methocel at a constant volume of 10 ml/kg. The untreated control group received a single oral dose of the vehicle only. A positive control group of 10 males was administered 240 mg/kg Cytoxan dissolved in sterile water by intraperitoneal injection. Following dosing, each male was mated weekly with two virgin females for eight consecutive weeks, so that each male was mated with a total of 16 females.

All mice were observed daily for mortality and signs of toxicity throughout the study. All males were weighed prior to treatment and weekly thereafter for nine weeks. Male mice were sacrificed and discarded on the last day of mating. Reproductive data were obtained following sacrifice of female mice at mid-generation.

The number and location of viable and non-viable fetuses, early and late resorptions, total implantations, and corpora lutea per dam were recorded. The thoracic and abdominal cavities and organs of the dams were examined for grossly evident morphological changes. Mutation rates, i.e., the proportion of early resorptions to all implantation, were evaluated weekly to determine susceptibility of the males germ cells during their maturation cycle.

Results:

No treatment-related effects were noted in general appearance, behavior or male body weights in any of the treatment groups for the duration of the study. Four deaths occurred: one male in the 2000 mg/kg test group during week 6 and three females. These females had been mated with different males one from eahc of the glyphosate test groups. The cause of death could not be determined at necropsy. Cytoxan produced a dominant lethal effect in the positive control group as evidenced by a statistically significant decrease in the number of viable fetuses and an increase in the proportion of early fetal deaths. The only statistically significant differences between the reproductive data of the glyphosate test groups and the untreated controls were decreases in the mean number of viable fetuses in the 800 mg/kg group during mating week 1 and in the 2000 mg/kg group during week 3. However, since no increase in early fetal deaths (i.e. early resorptions) accompanied these decreases, no mutagenic potential was attributed to glyphosate at these dosage levels.

Conclusion: Glyphosate did not produce a dominant lethal effect in mice at dosages up to 2000 mg/kg body weight.

Classification: Core-Minimum Data

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