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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: October 7, 1981

SUBJECT: EPA Reg.#524-308; Glyphosate: 3-Generation Rat Reproduction Study CASWELL#661A Accession#245409

FROM: William Dykstra, Toxicologist
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TO: Robert Taylor (25)
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Recommendations:

1. A NOEL could not be established for the study. The high incidence of focal tubular dilation in the kidneys of the male F_{3b} high-dose offspring were considered to be treatment-related. The registrant is required to examine histologically the kidneys of the low-dose and mid-dose male F_{3b} offspring in order to establish a NOEL for this effect.

Review:

1. A 3-Generation Reproduction Study in Rats with Glyphosate (Final Report; Biodynamics Project No. 77-2063; March 31, 1981).

   Test Material: Glyphosate; fine white powder (considered 100% active ingredient for dosing preparation); Lot No. XHJ-64

   CD (Sprague-Dawley derived) rats were administered Glyphosate continuously for three successive generations. Dietary concentrations of Glyphosate were adjusted weekly during growth, and between mating rest periods to acheive dose levels of 3, 10, and 30 mg/kg/day. Each generation (F_0, F_1 and F_2) consisted of 12 male and 24 female rats. Included in the study was a concurrent diet control group.

An experimental outline is shown below:

Group	Dose Level (mg/kg/day)	No. of Adults Initially Assigned to Mate F_0, F_1, F_2 Males	Females	No. of Matings per Generation F_0, F_1, F_2
I	0	12	24	2
II	3	12	24	2
III	10	12	24	2
IV	30	12	24	2

Each parent generation was mated to produce two litters. Offspring from the second litters of the F_0 and F_1 parents (F_{1b} and F_{2b} litters, respectively) were selected to be parents for subsequent generations. Offspring not included in the selection procedure and offspring from the first litter intervals of each generation (F_{1a}, F_{2a} and F_{3a}) were given a gross postmortem examination and discarded. Randomly selected offspring from the second litters of the F_2 generation (F_{3b} litters) were given a gross postmortem examination and selected tissues taken and saved. Subsequently tissues from control and high-dose F_{3b} offspring were evaluated microscopically (10/sex/group). Tissues from control and high-dose parent generations (F_0, F_1 and F_2) were also evaluated microscopically.

Parameters evaluated for each generation included: mortality, body weight and food consumption data (growth and rest period), in-life physical observation data, maternal body weights (gestation/lactation), reproduction-fertility indices (mating, pregnancy and fertility indices), litter data at parturition and organ weight data. Offspring from each litter interval were evaluated during a 21-day lactation period for growth, survival, sex distribution data and gross postmortem observations to include organ weight data (F_{3b} offspring only).

Tissues listed below taken from all parents (F_0, F_1 and F_2) and from 10/sex/group (chosen randomly) of the F_{3b} weanlings. All tissues preserved in 10% neutral buffered formalin. (Eyes and testes were placed initially in Bouin's solution).

adrenal (2)
aorta
bone and bone marrow (sternal)
brain (2 longitudinal sections)
eye (2) with optic nerve and Harderian gland
gonads
heart
intestine
  colon
  duodenum
  ileum
kidney (2)
liver (2 sections)
lung (section with mainstem bronchi)
lymph nodes (mesenteric)
mammary gland (right inguinal)
pancreas
pituitary
salivary gland
skeletal muscle (biceps femoris with right sciatic nerve)
skin
spinal cord (cervical and lumbar[5])
spleen
stomach
thyroid and parathyroid (attached to trachea and esophagus)
urinary bladder
uterus/prostate
gross lesions
tissue masses
thymus

The following organs were weighed from all parents sacrificed after weaning of the second litters and from eighty F_{3b} weanlings (10 males and 10 females per group) with tissues preserved.

adrenals
gonads
kidneys
brain
spleen
liver
heart
pituitary

Sections of all tissues listed above were prepared and examined microscopically from 10 male and 10 female animals from control and high-dose groups of the following:

Parents: F_0, F_1 and F_2
Offspring: F_{3b}

Any tissue masses observed in any animals were also examined.

Statistical analyses of the data were performed.

Results:

No treatment-related effect was evident in adult mortality data, body weight and food consumption data (growth and rest periods), and in-life physical observation data throughout the study (F_0, F_1, and F_2 generation).

Male and female mating indices and male fertility indices during both mating intervals of the F_0 generation were considered comparable between the control and treated groups. During the second mating interval of the F_0, pregnancy rates were lower than control in each of the treated groups; however, no indication of a dose-relationship was evident as the lowest pregnancy rate was seen in the mid-dose group. This reduction in pregnancy rate for the mid-dose group was not statistically significant. In the absence of a dose-response relationship the reduction in pregancy rate during this mating interval (F_{1b}) in the treated groups was not considered treatment-related.

In the F_1 generation, mating indices (males and females) for both litter intervals were comparable between the control and treated groups. It is note-worthy that for both mating intervals of this generation, mating indices for control and some treated groups were lower than normally encountered in multi-generation studies. The reason for the poorer mating performance in this generation in unclear but no treatment effect was indicated since mating indices were lowest in the control group. Pregnancy and male fertility indices for the first mating interval of the F_1 were comparable between the control and treated groups. During the second litter interval, pregnancy rates were lower than those seen for the first interval in control and treated groups. The lowest pregnancy rate was seen in the high-dose group; however, this difference from the control value was not statistically significant. Pregnancy rates for the low- and mid-dose groups, during the second mating interval, were considered comparable to control. Male fertility indices for this same mating interval were considered comparable between the control and treated groups.

In the F_2 generation mating indices for the treated groups were lower than control for each mating interval. During the first mating interval of the F_2 generation, the female mating indices were lower than control in each of the treated groups; however, only in the high-dose group was this difference from control statistical significant. The female mating index for the control group at this interval was 100% which is higher than normally encountered. The female mating indices observed for the control group in this study shown considerable variability ranging from 70.9 to 100%. The poor mating performance for the treated groups during the first mating interval is attributed to two males in each treatment group that did not mate either female in their mating unit (each mating unit is comprised of one male and two females).

During the second mating interval of the F_2 generation, male mating performance improved in the mid- and high-dose groups as both mid-dose males and one of two high-dose males that did not mate during the first mating interval, mated and impregnated at least one female. Male mating indices for the low-dose group remained unchanged as the same two males that did not mate during the first interval, failed to mate during the second interval.

Pregnancy and fertility indices for the treated groups were comparable to control for both litter intervals of the F_2 generation.

Over the entire study no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment.

No consistent adverse effects of treatment were evident in maternal weight data, gestation length, parturition data (number of live/dead pups at birth) or litter survival indices throughout the study. Concerning the offspring, no consistent treatment-related effects were indicated in sex distribution data, body weights, survival or gross postmortem findings. Also, no effect of treatment was evident in mean organ weight data (absolute and relative to body or brain weight) for randomly selected Day 21 F_{3b} offspring.

No adverse effect of treatment was evident in organ weight data for the F_0, F_1 generations and F_2 adult males. Mean spleen weights (absolute and relative to brain and body weights) were significantly higher than the control value in the F_2 mid-dose female group; however, mean spleen weight data for the low- and high-dose F_2 females were comparable to control values. In the absence of an effect on spleen weight in the high-dose F_2 female group, the change seen in spleen weight data for the mid-dose females was considered not biologically meaningful.

Gross postmortem evaluations of the adult generations of tissues from randomly selected F_0, F_1 and F_2 control and high-dose animals and F_{3b} control and high-dose offspring revealed a high incidence of focal tubular dilation in the kidneys of the male F_{3b} high-dose offspring.

Conclusion:

A NOEL could not be established for the study. The high incidence of focal tubular dilation in the kidneys of the male F_{3b} high-dose offspring were considered to be treatment-related. The registrant is required to examine histologically the kidneys of the low-dose and mid-dose male F_{3b} offspring in order to establish a NOEL for this effect.

Classification: Supplementary Data