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

OFFICE OF TOXIC SUBSTANCES

MEMORANDUM

DATE: July 29, 1980 Caswell No. 634

SUBJECT: Paraquat: review of 3 studies concerned with inhalation of paraquat and/or paraquat concentration in rat lung tissue. 239-2186

FROM: Krystyna K. Locke
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TO: Mr. T. Miller, Product Manager
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THRU: William L. Burnam, Acting Chief
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The following studies were reviewed:

1. "Three-week inhalation study in rats exposed to an aerosol of paraquat"; study no. ICI 254/7949; dated 8/8/79.

2. "Intrabronchial instillation of paraquat in rats: lung morphology and retention study"; no number and no date, but apparently a new study (latest references cited are dated 1978).

3. "Paraquat concentration in rat lungs following exposure to paraquat aerosols"' report no. CTL/P/460; dated 8/17/79.

Study (1) was classified as Core-Supplementary Data because hematology and clinical chemistry were not done, organs were not weighed, and tissues other than respiratory tract were not examined grossly or histopathologically. Based on the parameters tested (body wgt., food intake, clinical signs, and gross and histopathological examination of the respiratory tract), the No Observable Effect Level (NOEL) was 0.01 ug paraqart ion/liter of air.

Studies (2) and (3) were classified as Core-Minimum Data, in the category of SPECIAL TESTING (163.85-1). No lung damage was observed at paraquat levels of 10^{-7}g (0.1 ug) and lower (single instillations). Rats repeatedlly exposed to paraquat did not accumulate paraquat in the lungs between 5th and 15th exposure.

A detailed review of each study is submitted. It was stressed that the results in studies (2) and (3) should be interpreted with caution. ^{3}H, used in study (2), can exchange with body water and erroneous results are often obtained. The rats used in study (3) were only 26-32 days old and their ability to accumulate and to retain paraquat in lung tissue may not be the same as that of an older, or an adult rat.

1. Three-Week Inhalation Study in Rats Exposed to an Aerosol of Paraquat

Dated: 8-8-79. Study No. ICI 254/7949, Huntingdon Research Centre, Huntingdon, Cambridgeshire, England.

This study, designed to establish a no effect level, was carried out between 8-9-78 and 10-13-78.

Experimental Procedures

Spraque-Dawley CD rats, 156 M + 156 F, were exposed (whole body) to aerosolized paraquat ion for 6 hrs/day, 5 days/week, for 3 weeks (a total of 15 exposures). The dosage levels (ug parquat ion/liter) were as follows*: 0 (Group 1; Control), 0.01 (Group 2; Low dose), 0.10 (Group 3; Intermediate dose), 1.0 (Group 4; High dose, abandoned**), and 0.50 (Group 5; High dose, replacement). There were 32 M + 32 F rats in Group 1; 36 M and 36 F rats in each, Groups 2,3 and 4; and 16 M + 16 F rats in Group 5. Male rats were 26-30 days old on arrival and weighed 72- 96 g. The female rats were 28-32 days old and weighed 64-98 g. The test material was an aqueous solution of technical paraquat (aerosolized), containing about 40% (w/v) of paraquat ion. All particles were less than 2 um in diameter.

2

The following parameters were evaluated:

1. Body weight - rats were weighed individually twice/week. (Organs were not weighed).

2. Food intake - measured weekly.

3. Clinical signs - observed twice daily.

4. Lung paraquat analyses - determined for 4 M + 4 F rats after 5th and 15th exposure (Groups 1,2 and 3), and on the 1st, 2nd and 3rd day following the 15 exposures (Groups 2 and 3). These data were submitted in a separate report (CTL/P/460).

5. Gross and histopathological examinations - These examinations were restricted only to the respiratory tract (nasal passages, pharynx, tongue,larynx, trachea and lungs). At the terminal kill (after 15th exposure), 32 rats (16 M + 16 F) from Group I and 16 rats (8 M + 8 F) from Groups 2,3 and 5 were examined. At the recovery kill (2 weeks after end of exposure), 16 rats (8 M + 8 F) from Group 1,2,3 and 5 were examined. Group 4, the abandoned group (see ** below) was not examined.

*The average (+ S.D.) analyzed paraquat concentrations to which the animals were exposed are listed below.

Group	Paraquat ion (ug/l)
2 (Low dose)	0.012 (± 0.004)
3 (Intermediate dose)	0.112 (± 0.021)
4 (High dose, aborted)	1.280
5 (High dose, replacement)	0.487 (± 0.100)

**The original high-dose group (1.0 ug paraquat ion/l; Group 4) was abandoned because 28 M and 29 F rats died after the first exposure. This group was, therefore, replaced by Group 5 (0.5 ug paraquat ion/l of air). Results

1. Mortality - There were no deaths in Groups 1,2,3 and 5.

2. Body weight - There was a slight depression (2-4%, statistically significant: $p=<0.05$ and <0.01)* of weight gain in male rats from Groups 2 and 3. Because Group 5 was started one week later than the other groups and because there was no concurrent control, this group was not evaluated statistically. However, according to this submission, the animals in Group 5 "appeared to remain at a lower body weight than unexposed animals of the same age" (p. ii).

   *Statistical evaluation was done by the Williams test (1971/72), Biometrics 27:108 and 28:519.

3. Food intake - There was a slight reduction (4-6%) in food consumption for male rats in Groups 2 and 3 during the second and third weeks of exposure. Data from Group 5 were not analyzed statistically as there was no comparable control group.

4. Clinical signs - At 1 ug paraquat ion/l, 79% of rats died from respiratory failure following a single exposure. (This group was abandoned). At 0.5 and 0.1 ug paraquat ion/l, a few animals had brown staining around their noses and/or brown nasal discharge, lasting for 1-2 days after the first exposure. No clinical symptoms were observed at paraquat ion concentration of 0.01 ug/l.

5. Gross and histopathological examinations - Treatment-related changes were encountered in the following tissues:

   A. Pharynx
   Focal ulceration in 2 male rats at the 0.5 ug/l paraquat level, after 3 weeks of treatment.

B. Larynx
Extensive areas of ulceration, necrosis and acute inflammatory cell infiltration, with squamous keratinizing metaplasia and moderate/marked hyperplasia of adjacent epithelia-in all rats examined after 3 weeks of treatment (0.5 ug/l dose).

No ulceration or necrosis was observed in rats after a 2-week recovery period, following the 3 weeks of treatment.

At the 0.1 ug/l dosage level for 3 weeks, all of the 16 rats examined had squamous keratinizing metaplasia and/or hyperplasia of the epithelium. These changes were still observed in 11 rats after a 2-week recovery period.

C. Lungs - Aggregations of foamy macrophages in the bronchioles or alveoli, hypertrophy of the epithelium and thickened alveolar walls were present in most or all of the rats at the 0.5 ug/l level after 3 weeks of treatment. These changes were still seen after a 2-week recovery period. In addition, disruption of bronchiolar epithelium, adjacent to the macrophage aggregations, was noted.

Tissues other than the respiratory tract were not examined.

No observable effect level (NOEL)

Based on the parameters tested, the NOEL was 0.01 ug paraquat ion/liter of air.

Study Validation Category - Core-Supplementary Data. Reasons: 1)Hematology and clinical chemistry analyses were not done; 2)organs were not weighed; and 3)tissues other than the respiratory tract (pharynx, larynx, tongue, nasal passages, trachea and lungs) were not examined grossly and histopathologically.

2. Intrabronchial instillation of paraquat in rats: lung morphology and retention study.

Ian Wyatt, Adel W. Doss, Donald C. Zavala and Lewis L. Smith. ICI Limited, Cheshire, England (No Study No. or Date). EPA Acc. #241189

This is a copy of a paper which will be submitted for publication. The purpose of this study was two-fold: (1) to determine if the rat lung was as susceptible to instilled paraquat as the rabbit lung and (2) to determine the concentration of instilled paraquat which will produce histopathological damage.

Experimental procedures

(^{3}H methyl) paraquat dichloride (3 Ci/mmol) was instilled into the left bronchus of male adult rats (Wistar strain, body wgt.: 180-200g). In the distribution and retention experiments, single dosages of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} or 10^{-10} in 0.1 ml of saline were used. For each concentration of paraquat instilled, 3 rats were killed at 1,3,6,24,48 and 72 hours after dosing. Rats which were examined macroscopically and microscopically received nonradioactive paraquat. The concentrations used were the same as those listed above plus an additional one, 10^{-12} g in 0.1 ml of saline. Six rats per dosage were used, 3 of which were killed at 2 days after dosing and the remaining rats were killed at 14 days.

Results

1. Histological examination - No lung damage was observed at paraquat concentrations of 10^{-7} g (0.1 ug) and lower. At concentration of 10^{-6} g, some rats developed lung edema and macroscopic lesions. Two and 14 days after dosing with 10^{-5} g of paraquat, all of the lungs examined showed lesions and there was an evidence of polymorph infiltration.

2. Loss of paraquat from the lung - This loss was biphasic. The initial half-life (t 1/2) was less than one hour and was dose-independent. The secondary phase obeyed first order Kinetics and the t 1/2 was dose-dependent. For example, at paraquat concentration of 10^{-5} g, t 1/2 = 11 hours; at paraquat level of 10^{-10} g, t 1/2 = 76 hours.

3. Tissue distribution of paraquat - Lung, trachea, kidneys, urine and blood were examined. Only data for the 10^{-8} g of ^{3}H paraquat (10 ng) are reported. Fifteen minutes after the instillation of paraquat, the distribution of paraguat ion (%) was a follows: lung, 49.9; plasma, 34.6; urine, 1.7; kidneys, 1.5; and trachea, 1.2. The corresponding values for the 60-min. interval after instillation were 52.0, 12.5, 8.2, 0.5 and 1.5%, respectively.

4. After having discussed differences between their experimental procedures and those of Zavala and Rhodes[a], the authors could not conclude whether rabbit lungs or rat lungs were more sensitive to the instillation of paraquat.

[a]Zavala, D.C. and Rhodes, N.L. (1978). Chest 74:418-420. These investigators reported that the single instillation of 1pg of paraquat into the rabbit lung caused acute localized damage within 3 days.

All of the data on the distribution and retention of paraquat are expressed as means ± S.E.M. The histopathology data are discussed, but are not tabulated for individual animals.

Evaluation

In terms of the Core-concept, this study can be classified as Core-Minimum Data, in the category of SPECIAL TESTING (163.85-1).

Experiments involving tritium should be interpreted with caution. It is a common knowledge to those working with labelled compounds that ^{3}H is a problem-isotope because it can exchange with body water. Consequently, erroneous results are often obtained. The authors assumed that "there was no ^{3}H exchange from the ^{3}H paraquat whilst it was in the body of the animal". It is not a good scientific practice to make such assumptions, especially when alternatives are available. Why was not ^{14}C paraquat used? It is readily available commercially or it can be easily synthesized in the laboratory.

Although one objective of this study was to determine whether rat or rabbit was more sensitive to the instillation of paraquat, this question was never really answered. Obviously, an experiment with rabbits would have helped to clarify that problem.

1. M.H. Griffiths; Biochem. J. 108:731, 1968.

2. S.F. Contractor and B. Shane; Biochem. Pharmacol. 19:1669, 1970.

3. Paraquat Concentrations in Rat Lungs Following Exposure to Paraquat Aerosols.

Dated: 8/17/79. Report No. CTL/P/460, based on Study No. ICI 254/7949. Imperial Chemical Industries, Ltd. Cheshire, England.

This report is based on a 3-week inhalation study (15 6-hr exposures), identified as ICI 254/7949. The study was conducted by Huntingdon Research Centre, but the lung tissue was sent to ICI, Ltd., for paraquat analysis.

Experimental procedures

Paraquat concentrations in rat lungs were determined for 4 M + 4 F rats after 5th and 15th exposure (Groups 1,2 and 3), and on the 1st, 2nd and 3rd day of the recovery period (Groups 2 and 3). The exposure levels for Groups 1,2 and 3 were 0, 0.01 and 0.10 ug paraquat ion/liter, respectively. The determinations were done by the radioimmunoassay procedure (using ^{3}H-paraquat) which is detailed in this submission. The limit of detection was 0.1 ug paraquat ion/g of tissue. Full details of the inhalation study are given in a separate report, ICI 254/7949, dated 8/8/79.

No lung samples were available for analysis from Group 5 (0.5 ug paraquat ion/l). Reasons are not given, but this group was started one week later and was smaller (32 rats) than the other groups (72 rats/group).

8

Group 4 (1.0 ug paraguat ion/l) was abandoned because 79% of the rats died after the first exposure.

Results

Mean lung tissue concentrations of paraquat ion (ug/g wet wt. ± S.D.) are shown below.

Dosage ug/l	No. of exposures		Recovery period, days		
	5	15	1	2	3
0.01	0.11-0.12	0.09-0.13	0.01-0.10	ND*	ND*
0.10	2.08±0.46	1.66±0.35	1.34±0.25	0.65±0.09	0.35±0.12

*ND = Not detected.

These data show that paraquat does not accumulate in the lungs between the 5th and 15th exposure, and that it disappears rapidly after the termination of the exposure. During the recovery period for rats at the 0.1 ug/l dosage, paraquat concentration in the lungs fell by 20% during the first 24 hours and by 60% during the first 48 hours. According to the authors of this report, a repeated exposure of rats to paraquat aerosol does not result in a progressive accumulation of paraquat in the lungs.

The following points should be considered in the interpretation of this study:

1. Young rats were used in this study. The rats were 26-32 days old* when the study began and 47-53 days old when the study ended. Therefore, the ability of the lung tissue to accumulate and to retain paraquat may not be the same in a young and an adult rat.

2. Observations are based, essentially, on one exposure level (0.1 ug/l). Paraquat concentrations in the lungs at the 0.01 ug/l level equaled to, or were below, the detection limit for the analytical procedure used. Paraquat accumulation in the lungs at the 0.5 ug/l exposure level was not studied.

3. Paraquat accumulation in the lungs during the initial 5 days of exposure was not determined.

*The initial weight of the rats, 190 g, must be listed incorrectly in this report (p.4). The initial weight of rats in the 3-week inhalation study, on which this report is based, was 64-98 g (ICI/254/7949, p.2).

Study validation category: Core-Minimum Data, in the category of SPECIAL TESTING (163.85-1).

7/31/80