PP-1061
TXR-830

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

000830

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE:

SUBJECT: ORTHO Paraquat CL: 90-Day Feeding Study.
No. CTL/C/1027 Date: 2/17/81; EPA Accession No. 244873.
TOX Chem. 634

FROM: Krystyna K. Locke KKL 7/30/81
Toxicology Branch/HED (TS-769)

Krystyna K. Locke
7/30/81

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

THRU: Christine F. Chaisson, Acting Branch Chief
Toxicology Branch/HED (TS-769)

Study Submitted by: Chevron Chemical Company
Ortho Agricultural Chemicals Division
Richmond, California

Purpose: To support regulatory actions involving paraquat.

Summary:

The following observationns were made with regard to dogs fed ORTHO Paraquat CL (0, 7, 20, 60 and 120 ppm paraquat cation) for 13 consecutive weeks:

1. Two male and two female dogs from the 120 ppm level (3 mg paraquat ion/kg body weight) suffered from severe pulmonary toxic symptoms and had to be killed during the first month of the study. There were no other deaths, at any level tested.

2. The following treatment-related symptoms were observed at the 120 ppm level: weight loss, decreased food intake, increased lung weight, marked dyspnea, harsh rales, slow and/or irregular heart beat, large lesions in the lungs (alveolitis) and alveolar collapse. Clinical chemistry and urinalysis were unaffected. One of the four dogs killed early in the study showed increases in RBC numbers, hemoglobin level and packed cell volume.

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3. The following treatment-related symptoms were observed at the 60 ppm leve (1.5 mg paraquat ion/kg body weight): increased lung weight, alveolitis and alveolar collapse. There was a 5% weight loss ($p = < 0.01$) by the female dogs, but it was considered to be treatment-unrelated. Other parameters tested were unaffected.

4. The 20 ppm level (0.5 paraquat ion/kg body weight) was considered by the testing laboratory to be a no-effect-level. The only symptom noted at this level was a 12% weight loss ($p = < 0.01$) by the female dogs. This loss was dose-related and was not attributed to paraquat. There was no obvious reason for this weight loss.

5. At the 7 ppm level (0.[illegible] mg paraquat ion/kg body weight), paraquat had no effect on any of the parameters tested.

6. Core classification: Minimum, upgraded from Supplementary (there were only three dogs/sex/level and the study lasted only 13 weeks).

7. No Observable Effect Level (NOEL). This reviewer considers 20 ppm (0.5 mg paraquat ion/kg body weight) to be a NOEL, based on the following parameters tested: visual appearance, behavior, food consumption, hematology , clinical chemistry, urinalysis, ophthalmology, auscultation, organ weights, gross necropsy, histopathology, and body weights.

   The 12% weight loss at this level by the female dogs, although statistically significant ($p = < 0.01$), is a small weight loss. This weight loss is dose-unrelated and apparently treatment-unrelated, since all of the other parameters tested were negative.

   One-year dog feeding study was started in January, 1981 and will eventually replace this 13-week study, as far as the toxicity data requirements are concerned.

8. Typographical Omissions:

   It should be indicated in Table 4 (p. 39) and Table 5 (pp. 40-41) that the data reported for the 120 ppm level, for test weeks 6 and 12, represent one male and one female dog. These data are not group mean values.

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Paraquat Thirteen Week (Dietary Administration) Toxicity Study in Beagles
EPA Accession No. 244873

ICI Study No. PD 0394
Chevron's Study No. CTL/C/1027
Testing Laboratory : Hazleton Laboratories Europe Ltd.
Harrogate, England

Test started: 3/25/80
Test terminated: 6/24/80

Report prepared by: D. B. Sheppard, B. Sc., Toxicologist
Date of issue: 2/17/81
Chief Pathologist: J. R. Glaister, BVM and S, DVSM, PhD, MRCVS

This study was initially designed to be a preliminary dose range-finding study of 6 weeks duration, in order to determine dose levels for an one-year dog feeding study started in January, 1981. However, when the study was already in progress, the sponsor informed the testing laboratory "that there was a regulatory requirement for a 13 week dietary toxicity study on paraquat, the report of which should be available by early 1981". The study was, therefore continued for 13 weeks, with the knowledge that "the animals numbers in each dose level are less than is current practice for a 90 day study". (Memo from L. Stelzer, Manager, Registration and Regulatory Affairs, Chevron Chemical Company; to Robert J. Taylor, PM 25, EPA).

Experimental Procedures

Beagle dogs from the ICI breeding colony, 3 males and 3 females/dose level, received paraquat dichloride for 13 weeks at the following levels (expressed as paraquat cation): 0, 7, 20, 60 and 120 ppm. The test material, added to the diet, was the technical grade aqueous solution containing 32.2% w/w of paraquat cation. The dogs, aged 7-8 months at the start of the study, were given 400 g of the appropriate diet mix every morning. Any remaining diet was removed and weighed the following morning (about 24 hours later). Diets were prepared in 20 kg batches every two weeks, were checked for homogeneity with regard to paraquat concentration, and were stored at room temperature. Paraquat in diets was checked for stability on days 1, 7 and 14. Diets were checked for paraquat concentration during weeks 1, 3, 5, 7, 9, 11 and 13 of the study. The basic diet was laboratory Diet A, expanded and reground, manufactured by B. P. Nutrition (England) Ltd., Witham, Essex. A certificate was supplied with each batch used detailing analysis of dietary constituents and contaminants, including heavy metals, aflatoxins, organic compounds and micro-organisms. Water was available for the animals via an automatic

drinking system. Water was routinely analyzed for specified contaminants by the local water authority and also periodically by the Hazleton Laboratories. The following parameters were evaluated:

1. Clinical Condition and Behavior:

   All animals were observed periodically throughout the day by an experienced technician. In addition, a detailed clinical examination was made by a veterinarian before the treatment and after 6 and 12 weeks of treatment.

2. Body Weight:

   Individual body weights were recorded weekly on the same day and before sacrifice.

3. Food Consumption:

   Individual food consumption was recorded daily.

4. Hematology:

   The following determinations were made on all dogs before the start of treatment and after 3, 6 and 12 weeks of treatment:

   hemoglobin
   mean cell volume
   packed cell volume
   total white blood cell count
   differential white cell count*
   platelet count*
   prothrombin*
   activated partial thromboplastin time*
   RBC
   mean cell hemoglobin

   * Not done after 3 weeks.

5. Clinical Chemistry:

   The following determinations were made on heparinized plasma before the start of treatment and after 6 and 12 weeks of treatment:

   glucose*
   cholesterol
   blood urea nitrogen*
   bilirubin
   total protein
   protein electrophoresis
   GOT*
   sodium
   potassium
   calcium
   chloride
   alkaline phosphatase
   creatine phosphokinase
   GPT*

   * Done also after 3 weeks of treatment.

All blood samples were obtained by jugular venepuncture after an 18-hour fasting period. The determinations were made on all dogs. Serum lactic dehydrogenase was not determined.

6. Urine Analysis:

Urine samples were collected from all dogs, by direct catheterization of the bladder, once before the start of dosing, after 3 and 6 weeks of treatment, and preterminally. The following parameters wree examined:

pH
protein*
ketones*
bilirubin
urobilinogen*
microscopy of spun deposits*
specific gravity
glucose*
blood*
reducing agents*

* Semi-quantitative assessment only.

7. Ophthalmology:

The eyes of all dogs were examined with a hand-held Keeler direct ophthalmoscope, once before the start of dosing and then after 6 weeks of treatment, and preterminally. The eyes were treated with 1% tropicamide about 15 minutes before examination.

8. Auscultation:

A detailed examination by auscultation was performed on all dogs before the start of dosing, after 6 weeks of treatment and preterminally.

9. Organ Weights:

The following organs from all dogs were weighed prior to fixation:

brain
pituitary
spleen
thymus
lungs*
liver
adrenals
kidneys*
gonads
heart
thyroids

* Lungs and kidneys only were weighed in the 2 male and 2 female dogs from the 120 ppm level which were killed in extremis. The dogs were killed between experimental days 16 and 23 when the 6-week dietary study protocol was in operation.

10. Necropsy:

Necropsy was performed on all dogs (26) killed at the end of the study and on those four killed in extremis. The dogs were killed over the period of four days, 5 on 6/24 and 7 on 2/25, 6/26 and 6/27/80.

11. Histology:

The following tissues were examined:

adrenals
aorta
bone marrow
brain
cecum
colon
duodenum
eyes
gall bladder
heart
ileum
jejunum
kidneys
lungs
lymph nodes
skeletal muscle
all unusual lesions
tongue
uterus (corpus and cervix)
esophagus
ovaries
pancreas
pituitary
prostate
rib marrow
sciatic nerve
skin
mammary gland
spinal cord
spleen
stomach
salivary gland
testes
trachea
thymus
thyroids
urinary bladder

Results

1. Mortalities:

Four dogs, 2 male and 2 female, from the 120 ppm level had to be killed between 16 and 23 days of treatment. These dogs suffered from marked dyspnea, harsh rales, slow and/or irregular heart beat, and weight loss.

2. Clinical Condition and Behavior:

Apart from those dogs killed in extremis and another dog (No. 76M; 20 ppm) who had fever on two occasions, all dogs appeared normal throughout the study. Dog No. 76M suffered from fever, lack of appetite and enlarged submandibular lymph nodes during week 3 of the study. He was given Streptopen for 3 days and quickly recovered. He was then sick again with the same symptoms during week 7 of the study. He recovered quickly after treatment with Engemycin and Parentrovite. This illness had apparently no relationship to paraquat treatment.

3. Body Weight:

These data are tabulated for individual animals and as the group mean weekly body weights/sex, for 11 weeks before the test and for 13 weeks of the treatment period. The group mean body weights/sex are also represented graphically.

Paraquat did not affect body weights of male dogs at the 7, 20 and 60 ppm level. There were 1-4% dose-unrelated weight gains or losses, when the experimental groups are compared with controls during the test week 13. The body weights of male dogs at the 0, 7, 20 and 60 ppm level remained constant during the 13-week test period. The only surviving dog at the 120 ppm level lost 0.6 lb. (4%) of weight during the 13 weeks of treatment with paraquat.

There was a 4, 12 and 5% weight loss for female dogs at the 7, 20, and 60 ppm level, respectively, when the experimental groups are compared with controls during the test week 13. Because the weight loss at the 20 ppm level is greater than that at the 60 ppm level, it is probably treatment-unrelated.

The weight gain of female dogs during the 13-week test period was 1.14 lbs. (10%), 0.5 lb. (4%) and 0.3 lb. (3%) at dose levels of 0, 7 and 60 ppm, respectively. The weight loss of female dogs at the 20 ppm level was only 2% during that time. The only surviving dog at the 120 ppm level lost 0.9 lb. (8%) of weight during the 13 weeks of treatment with paraquat.

4. Food Consumption:

These data are tabulated for individual animals and as the group mean weekly food consumption/sex, for 11 weeks before the test and for 13 weeks of the treatment period. The group mean weekly food consumption /sex is also represented graphically. The group mean paraquat intake in mg cation /kg body weight/day/sex is tabulated on weekly basis for 13 test weeks.

There were no effects on food intake at any of the levels employed, except for one surviving female at the 120 ppm level. Each dog received 400 g of food per day and ate all of it. The surviving high dose female consumed 33-78% of her food allowance during the last six weeks of the test. The intake varied on weekly basis and it was not a progressive decrease in consumption.

5. Hematology and Clinical Chemistry:

These data are reported for individual animals and as group averages/sex. There were no treatment-related changes in any of the parameters examined. The variations seen in the treated dogs were similar to those observed in the controls and were, therefore, considered normal. Dog No. 86M at the 120 ppm level, killed on day 16 of the study, showed increases in red blood cell numbers, hemoglobin level and packed cell volume.

6. Urine Analysis:

These data are reported for individual animals. There were no findings that could be attributed to paraquat treatment.

7. Ophthalmology:

Congestion of retinal blood vessels was observed in two dogs at the 120 ppm level and in one dog in each the 7 ppm and the 20 ppm group. None was seen at the 60 ppm level. Other observations were as follows: Pinpoint vacuoles in cornea, in both eyes (one dog in the control group) and focal keratitis or corneal ulcer (two dogs at the 60 ppm level). These findings did not appear to be treatment-related.

8. Auscultation:

All dogs were normal at the pretest auscultatory examination. At the week 6 examination, the two surviving dogs in the 120 ppm group and three dogs in the control group exhibited increased respiratory sounds. At the week 12 examination, 15 dogs, 2-4 dogs from each test group and three dogs from the control group, showed increased respiratory sounds. These respiratory sounds were, therefore, not considered to be treatment-related.

9. Organ Weights:

These data are reported as individual and group mean absolute and relative weights.

Absolute and relative lung weights were increased 75-76% and 92-104% respectively, in all high dose (120 ppm) dogs compared to the controls. Absolute and relative lung weights were also increased in two dogs treated at 60 ppm, male dog No. 77 and female dog No. 97. In the case of the male dog, absolute and relative lung weights were increased 39% and 44%, respectively. The corresponding values for the female dog were 41% and 56%. the lung weights of all other treated dogs were similar to the controls. Paraquat did not affect the weights of other organs examined.

9. Pathology:

Gross necropsy and histopathology data are reported for individual animals and are also tabulated as "Incidence of pathology findings" (Table 7.5.1, pp. 162-165; enclosed with this review). The individual reports do not contain the time of death of an animal, but these data are presented separately under EXPERIMENTAL PROCEDURES, p. 15.

Large treatment-related lungs lesions, classified as alveolitis, were found in all dogs at the 120 ppm level and in 5 out of 6 dogs used at the 60 ppm level. The alveolitis usually consisted of a mixture of exudative and proliferative reactions resulting in alveolar collapse, distortion, and interstitial hypercellularity. Two males and two females in group 5 (120 ppm) had to be sacrificed during the study days 16-23 because of the severity of the lung lesions. Alveolitis was not observed at other dosage levels tested.

The following findings were seen at all dosage levels tested and in the controls: pneumonitis, pulmonary perivascular and peribronchiolar leucocyte accumulation, and hepatic periportal and intralobular foci. According to Dr. Louis Kasza, Pathologist, Toxicology Branch, these findings are indications of poor animal husbandry.

It is not clear why medullary mineralization was observed in all but one dog used in this study. According to Dr. Kasza, medullary mineralization is common in old dogs. However, these dogs were only 8-11 months old at the time of the sacrifice and obviously were not old.

Small pituitary cysts were seen in several animals, at all dose levels and in the controls. According to Dr. Kasza, these cysts in low grade occurrence are common in dogs.

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7.5 SUMMARY TABLES

Table 7.5.1 Incidence of pathology findings (13 week)

Tissue/observation \ Group no. / Sex	1 0 ppm		2 7 ppm		3 20 ppm		4 60 ppm		5 120 ppm	
	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	*M(3)	*F(3)
LUNGS										
alveolitis							2	3	3	3
pneumonitis	2	2	2	1	2	2				
bronchiolitis	1		1		1	1				1
interstitial fibrosing alveolitis	1		1				1			
granuloma	1				2		1			1
perivascular lymphoid hyperplasia/leucocyte accumulation	2	2	3		1	3	1			
peribronchiolar leucocytes	2	3	3	2	2	2	1			
peribronchiolar pigment cells	1	1								
alveolar bone				1						
KIDNEYS										
swollen cortical tubules							1		1	1
regenerating cortical tubules						1				
medullary mineralisation	2	3	3	3	3	3	3	3	3	3
nephritis		1	1							1
pyelitis		1	1	1			1		2	1
diminished fat					2				1	

* Two dogs were necropsied before study termination.

Table 7.5.1 (continued) Incidence of pathology findings (13 week)

Tissue/observation \ Group no. / Sex	1		2		3		4		5	
	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	*M(3)	*F(3)
ADRENALS										
capsular mineralisation					1					
cortical lipid vacuolation									1	
ARTERY (adjacent to aorta)										
arteritis			1							
BRAIN										
perivascular lymphoid foci cell				1						
COLON/RECTUM										
congested lymphoid follicles			1					1		
JEJUNUM										
nematodes					1		1	1		1
LIVER										
periportal leucocyte foci	2	3	2	3	1	3	1	3	1	1
intralobular leucocyte foci	2	2	2	2	3	3	2	2	2	1
LYMPH NODES: BRONCHIAL										
erythrophagocytosis										1
LYMPH NODES: MESENTERIC										
granulomas			1		1	1	1			

* Two dogs were necropsied before study termination.

Table 7.5.1 (continued) Incidence of pathology findings (13 week)

Tissue/observation \ Group no. / Sex	1		2		3		4		5	
	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	*M(3)	*F(3)
PITUITARY										
cyst/cysts in pars distalis	2	2	2		1	2	1	1	1	1
PROSTATE										
prostatitis/interstitial lymphoid cell foci	1		2		2					
REPRODUCTIVE TRACT										
pro-oestrus		1								
SKIN										
folliculitis			1		1					1
SPLEEN										
arteritis			1							
congestion			1		2			1		1
fibrosis			1						1	
pale raised areas										1
STOMACH										
myositis							1			
fundic mucosal mineralisation			1		1					
SUBMAXILLARY GLAND										
interstitial lymphoid cell foci						1				

* Two dogs were necropsied before study termination.

Table 7.5.1 (continued) Incidence of pathology findings (13 week)

Tissue/observation \ Group no. / Sex	1		2		3		4		5	
	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	M(3)	F(3)	*M(3)	*F(3)
TESTES										
atrophy			1				1			
THYMUS										
involution							1		1	2
TONGUE										
glossitis									1	
URINARY BLADDER										
cystitis		1				2				1
congestion/submucosal lymphoid follicles						1		1		1
haemorrhage										1
lymphoid hyperplasia						2				

* Two dogs were necropsied before study termination.