RD-584
TXR-456

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

004968

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

MEMORANDUM

Subject : D P XF6025 also known as Classic

From : Alex Arce
Toxicology Branch Reviewer (T S 769) 3-5-86

To : Taylor / Walters P M 25
Hazard Evaluation Division (T S 767)

Thru: Clint Skinner Ph. D. 3-6-86
Section 111 Head

Theodore Farber Ph. D. 3/6/86
Chief , Toxicology Branch (T S 769)

Action Requested

Review of Submitted data .

This is additional data in support of the Petition .

Product Identification Number 5 F 3186 / 352 UGA

Acc # 073802-3-4 Record # 159280/159281

Caswell # 193 B

Recomendations

The submitted studies has been reviewed and the results are as follows :

One Year dog feeding HLR No 232-85 - Core Guidelines

NOEL = 250 ppm

LEL= 1,500 ppm (Increase in alkaline phosphatase - male; decrease in erythrocytes , increase in leukocytes male and female ; increse in mean relative liver weight -female

Chronic Feeding Study -rat HLR422-85 - Core Minimum

NOEL= 250 ppm

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LEL = 2,500 ppm ; decrease in body weights in male and females

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Metabolism - rat Inconclusive AMR 220-84

The study is inconclusive and is graded as supplementary data , the study has to be repeated due to the following :

Only two rats /sex /group instead of five .

No individual animal data is submitted .

No individual animal weights make doubtful calculations for dosing

2 generation reproduction - rat CORE Minimum HLR 422-85

NOEL = 250 ppm

LEL = 2,500 ppm

Body weight reduction (significant)

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EPA: 68-02-4225
DYNAMAC No. 1-061-B1,2
March 5, 1986

DATA EVALUATION RECORD

DPX-F6025 (INF-6025)

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Stadler, J. C. Long-term feeding and two-generation, four-litter reproduction study in rats with INF-6025. (Unpublished study No. HLR 422-85 prepared by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. DuPont de Nemours and Co., Wilmington, DE; dated August 26, 1985.) Accession Nos. 073803-073804.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-5-86

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1. CHEMICAL: DPX-F6025; INF-6025; F-6025; chlorimuron ethyl; ethyl 2-[[[[(4-chloro-6-methoxy-pyrimidin-2-yl)amino]carbonyl]amino]sulfonyl] benzoate.

2. TEST MATERIAL: INF-6025 contained 96% active ingredient.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Stadler, J. C. Long-term feeding and two-generation, four-litter reproduction study in rats with INF-6025. (Unpublished study No. HLR 422-85 prepared by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. DuPont de Nemours and Co., Wilmington, DE; dated August 26, 1985.) Accession Nos. 073803-073804.

5. REVIEWED BY:

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Date: 3-5-86

Alex Arce
EPA Reviewer

Signature:
Date: 2-4-86

Clint Skinner, Ph.D., D.A.B.T.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. The NOEL and LOEL for adult toxicity are 250 and 2500 ppm INF-6025, respectively, based on significant body weight decreases reported at 2500 ppm. No adverse effects were reported for fertility and gestation indices even at the highest dietary level tested (2500 ppm).

The NOEL and LOEL for pup toxicity are 25 and 250 ppm INF-6025, respectively, based on significant body weight decreases and organ weight changes reported at 2500 ppm and on significant increases in the incidences of histopathological findings at 250 and 2500 ppm.

B. Some individual pup data for body weights, counts, and viability of F_{1a} pups were not presented, although data for F_{1b} pups from the 2500-ppm group were presented twice in the study report appendices. In addition, histopathological findings for F_{2b} pups were not adequately described to allow a definitive assessment of possible compound-related effects. Since these deficiencies did not preclude the assessment of the reproductive toxic potential of the test material, this study is classified Core Minimum.

8. RECOMMENDATIONS: We recommend that selective stains be used to characterize the histopathological findings reported for livers of F_{2b} pups in the 250- and 2500-ppm groups or that the testing laboratory submit additional data to substantiate the author's conclusion that these liver findings were not compound related.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material, INF-6025, Haskell No. 14,851, contained 96% active ingredient. Animals in this study were continuously fed diets containing 0 (control), 25, 250, or 2500 ppm of the test material.

2. The two-generation reproduction phase was part of a 2-year feeding study in rats. Crl:CD(SD)BR rats, approximately 22 days old, were received from Charles River Breeding Laboratories, Kingston, NY. Rats were housed in a temperature-, humidity-, and light-cycle-controlled room.

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[1] Only items appropriate to this DER have been included.

Animals were given food (Certified Purina Laboratory Chow No. 5002) and water ad libitum. Following an acclimatization period of 15 days, rats of each sex were assigned to study groups by computerized randomization.

Animals were housed one per cage prior to mating and exposed for 96 days to the corresponding test diets. Twenty rats per group per sex were selected to become F_0 parents in the reproductive study. Males and females from the same exposure levels were cohabitated on a 1:1 basis for 15 days, and females were checked daily for presence of copulatory plugs. At the end of gestation, females were checked twice daily for the birth of F_{1a} pups. The number of live and dead pups were counted soon after birth and at days 1, 4, 12, and 21 postpartum. Pups were culled on day 4 to reduce litters to no more than 10 pups (with an approximate equal number of males and females). Litter weights were obtained at 24 hours and 4 days postpartum. Pups were weaned, weighed, sexed, and sacrificed, but not examined, at 21 days of age.

Each F_0 female was allowed to rest for 10 days after F_{1a} pups were weaned, and then mated with a different F_0 male. The resulting F_{1b} pups were handled as described for the F_{1a} generation until weaning, at which time 20 male and 20 female pups were randomly selected from each dietary level to become parents of the F_2 generations. After a 90-day exposure to the test diets, the F_{1b} animals were mated to produce F_{2a} pups (which were sacrificed at weaning without pathological evaluation), and subsequently mated once again to produce the F_{2b} pups. Ten male and 10 female pups were selected at weaning from each group for gross and histopathological evaluations. Liver, brain, kidneys, and testes were weighed; in addition to these organs, several other tissues from control and high-dose animals were processed (including female reproductive organs) for histological examination; in low- and mid-dose animals, only brains, livers, and gross lesions were examined histologically. Data were analyzed using appropriate statistical methods; differences between means were considered significant at the $p < 0.05$ level.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Diet Analyses: Results from analyses of diet samples obtained on test days 0, 28, 483, 609, and 728 indicate that the mean concentrations of test diets were within 6% of nominal concentrations. The homogeneity of test diets was reportedly acceptable and, therefore, the study author considered that INF-6025 was homogeneously distributed in the test diets.

B. The study author reported statistically significant reductions in mean body weights for the group of approximately 80 F_0 males and 80 F_0 females exposed to 2500 ppm in the 2-year study. Results for the premating body weights of the 20 F_0 animals per group per sex in the reproduction phase of the study were not discussed in the text; however, individual animal data were presented for these animals in Appendix C of the study report. Premating body weights for F_{1b} males and females were statistically comparable for all groups except for the significantly higher body weights and weight gains reported for F_{1b} females in the 25-ppm group (Table 1). Body weights and weight gains of F_{1b} females in the 2500-ppm group were slightly less than control values, but these differences were not statistically significant (Table 1).

Food consumption data for F_{1b} males suggest no compound-related effects in this parameter; however, the study author reported a slight (but not significant) reduction in food consumption for F_{1b} females fed 2500 ppm INF-6025. Food efficiency values for males and females in the dose groups were comparable to their corresponding controls. These data and group mean intakes of INF-6025 in F_{1b} animals are presented in Table 2.

No compound-related effects were reported for mortalities or animal appearance and behavior.

No compound-related effects on fertility indices, gestation indices, or pup viability during the 21-day lactation period were reported for any generation. Some values were reduced when compared with controls (for example, fertility indices of the first and second matings of F_0 parents in the 250-ppm groups), but these changes were not considered statistically or biologically significant (Table 3).

Significant reductions in body weights of F_{1a} pups and their mothers were noted on postpartum day 21 for the 2500-ppm group when compared with controls; body weights of these pups were slightly reduced at 24 hours but this difference was not statistically significant. No differences were reported for the number of F_{1a} pups throughout lactation (Table 4). The only statistically significant change in F_{1b} pup body weights was a reduction in the weight of female pups from the 2500-ppm group at day 21 when compared with controls (Table 4). Slight (nonsignificant) reductions in male pup and maternal weights were also reported for this group at the end of lactation.

Significant reductions in mean pup body weights were noted for F_{2a} males and females at day 21 in the 2500-ppm group when compared with controls (Table 4). In addition, the body weights of the mothers of these pups were also significantly reduced when compared with controls. Total litter weights for this group were significantly reduced at 24 hours; this change was associated with

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TABLE 1. Effects of INF-6025 on Premating Body Weights and Body Weight Gains in Rats

	Dietary Level (ppm)			
	0	25	250	2500
		Male F_0 Rats[a]		
Day 0	153.5± 8.5[b]	150.0± 7.5	151.0± 9.9	147.6± 7.6
Day 91	525.3±49.9	500.7±49.8	494.1±39.1	491.8±34.7
Weight change (0-91)	371.8±46.8	350.7±46.8	343.1±34.9	344.1±30.3
		Female F_0 Rats		
Day 0	127.5± 9.1	126.6± 7.1	125.0± 7.5	122.8± 8.6
Day 91	269.3±29.2	279.3±22.3	281.5±34.5	251.1±35.6
Weight change (0-91)	141.8±24.5	152.7±20.8	156.5±32.1	128.3±30.5
		Male F_{1b} Rats		
Day 0	180.6±15.8	185.7±22.9	174.2±31.6	170.3±21.3
Day 91	554.0±52.7	576.0±54.7	569.1±51.6	552.5±56.4
Weight change (0-91)	373.4±48.0	391.5±40.4	394.9±51.7	382.2±44.8
		Female F_{1b} Rats		
Day 0	141.8± 8.27	145.5±12.8	133.3±22.6	136.3±12.9
Day 91	277.6±26.7	305.6±46.9*	285.4±36.6	262.5±19.4
Weight change (0-91)	135.9±21.8	160.6±38.6*	152.1±33.6	126.1±18.0

[a]Values for F_0 rats were calculated by reviewers from individual animal data.

[b]All values represent group mean (g) ± SD based on approximately 20 animals.

*Significantly different from control value ($p \leq 0.05$).

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TABLE 2. Food Consumption, Food Efficiency, and Intake of INF-6025 in F_{1b} Rats During the Premating Period

	Dietary Level (ppm)			
	0	25	250	2500
		Males		
Food Consumption	27.9[a]	29.8	28.4	29.1
Food Efficiency	0.147[b]	0.145	0.153	0.144
Intake of INF-6025	0.0[c]	1.7	17.0	177.0
		Females		
Food Consumption	19.5	21.1	19.5	18.8
Food Efficiency	0.076	0.083	0.086	0.074
Intake of INF-6025	0.0	2.2	21.0	214.0

[a]Group mean (g) for days 0-91.

[b]Group mean (g weight gain/g diet consumed) for days 0-91.

[c]Group mean (mg INF-6025/kg body weight/day) for days 0-91.

TABLE 3. Effects of INF-6025 on Fertility, Gestation, and Viability Indices in Rats

	Dietary Level (ppm)			
	0	25	250	2500
	F_0 Mating: F_{1a} Pups			
Fertility Index (%)[a]	80	95	58	95
Gestation Index (%)[b]	100	100	100	100
Pup Viability (%)				
Day 0	98	98	98	99
Days 0-4	98	100*	99	97
Days 4-21[c]	94	100	100	93
	F_0 Mating: F_{1b} Pups			
Fertility Index (%)	78	74	61	90
Gestation Index (%)	100	100	100	94
Pup Viability (%)				
Day 0	99	99	98	94
Days 0-4	100	100	98	94
Days 4-21	100	100	100	98

(Continued)

[a] $\frac{\text{No. litters delivered}}{\text{No. females mated}} \times 100$; indices are based on 18-20 mated females per group.

[b] $\frac{\text{No. litters with one or more live pups}}{\text{No. litters delivered}} \times 100$.

[c] Defined as lactation index by the study author.

* Significantly different from control value ($p \leq 0.05$).

TABLE 3. Effects of INF-6025 on Fertility, Gestation, and Viability Indices in Rats (Continued)

	Dietary Level (ppm)			
	0	25	250	2500
	F_0 Mating: F_{1a} Pups			
Fertility Index (%)[a]	85	68	80	80
Gestation Index (%)[b]	100	100	94	100
Pup Viability (%)				
Day 0	99	99	92	99
Days 0-4	99	96	99	99
Days 4-21[c]	100	100	97	100
	F_0 Mating: F_{1b} Pups			
Fertility Index (%)	75	63	80	85
Gestation Index (%)	100	100	100	100
Pup Viability (%)				
Day 0	98	97	100	95
Days 0-4	99	100	99	93
Days 4-21	100	100	100	94

(Concluded)

[a] $\frac{\text{No. litters delivered}}{\text{No. females mated}} \times 100$; indices are based on 18-20 mated females per group.

[b] $\frac{\text{No. litters with one or more live pups}}{\text{No. litters delivered}} \times 100$.

[c] Defined as lactation index by the study author.

TABLE 4. Effects of INF-6025 on the Number and Body Weight of Rat Pups

	Dietary Level (ppm)			
	0	25	250	2500
			F_{1a} Pups	
No. at 24 hours	10.7±3.2[a]	11.3±2.7	11.6±2.7	11.4±2.2
Body weight (g) at 24 hours	7.3±1.5	7.2±0.8	6.7±0.8	6.5±1.0
No. at day 21	8.4±3.1	9.3±1.4	9.5±1.3	9.0±2.4
Body weight (g) at day 21				
Male pups	49.9±7.7	50.9±6.2	49.4±3.5	40.1±5.4*
Female pups	47.0±8.3	48.9±6.4	47.2±3.9	38.1±5.4*
			F_{1b} Pups	
No. at 24 hours	11.4±3.1	12.6±3.6	11.8±4.0	10.2±4.5
Body weight (g) at 24 hours	6.8±0.7	6.9±0.6	7.0±0.8	6.7±0.9
No. at day 21	9.4±1.3	9.4±2.1	8.9±2.2	8.2±3.1
Body weight (g) at day 21				
Male pups	48.9±5.4	50.9±4.6	51.7±5.1	45.6±6.5
Female pups	47.5±4.6	49.4±4.1	50.5±5.8	42.0±8.4*

(Continued)

[a]Values represent group means ± SD.

*Statistically different from control value ($p \leq 0.05$).

TABLE 4. Effects of INF-6025 on the Number and Body Weight of Rat Pups (Continued)

	Dietary Level (ppm)			
	0	25	250	2500
			F_{2a} Pups	
No. at 24 hours	12.9±3.0[a]	10.5±4.5	11.5±4.3	11.1±2.5*
Body weight (g) at 24 hours	7.0±0.9	7.0±1.4	7.2±0.7	6.9±1.4
No. at day 21	9.6±1.7	8.2±2.6*	8.8±3.1	9.5±1.3
Body weight (g) at day 21				
Male pups	47.5±4.0	51.0±6.4	48.1±5.9	41.2±6.0*
Female pups	46.0±4.2	49.1±4.9*	46.5±5.3	39.8±5.6*
			F_{2b} Pups	
No. at 24 hours	13.3±3.0	13.8±2.8	13.6±2.6	12.2±3.8
Body weight (g) at 24 hours	6.9±0.5	6.7±0.4	7.0±0.7	6.3±1.0*
No. at day 21	9.7±1.0	9.8±0.6	9.8±0.8	9.2±2.5
Body weight (g) at day 21				
Male pups	54.5±4.3	53.4±3.5	51.5±4.7	43.3±4.2*
Female pups	50.9±3.2	51.2±2.9	49.7±4.6	41.9±4.0*

(Concluded)

[a]Values represent group means ± SD.

*Statistically different from control value ($p \leq 0.05$).

significant reductions in the number of pups per litter in this group when compared with controls. No compound-related changes were reported for F_{2a} pups in the 250- and 25-ppm groups.

Significant body weight reductions were noted for litters and pups from the 2500-ppm group throughout gestation. Maternal body weights at postpartum day 21 were also significantly reduced for this group when compared with controls. Pup body weights in the other dose groups were comparable with controls (Table 4).

Significant changes in organ weights from F_{2b} pups were noted only in the 2500-ppm group when compared with controls. Absolute liver and kidney weights were reduced, and relative brain and testes weights were increased in males. Among female pups, relative brain and liver weights were increased and absolute kidney weights were decreased (Table 5). Organ weights for F_{2b} pups in other dose groups were comparable to controls.

The study author reported statistically significant effects in cerebellar and hepatic histopathological findings in F_{2b} pups from the 2500-ppm group. The cellular changes in the internal granular and external germinal layers of the cerebellum were considered compound related and associated with possible nutritional effects of this dietary level. Statistically significant increases in hepatic cytoplasmic vesiculation reported for the 250- and 2500-ppm groups were reportedly artifactual and attributed to the order of pup sacrifices.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Adult and pup body weight reductions and histological changes in the cerebellums of pups from the 2500-ppm group were considered compound-related effects. No compound-related effects were noted at 250 or 25 ppm.

B. A quality assurance statement was signed and dated on August 19, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Results from chemical analyses suggest that the homogeneity, stability, and concentration of test diets were acceptable.

Group mean body weights and body weight gains for F_0 parents during the 90-day premating period were not presented in the study report; however, individual weights were included in Appendix C. Our calculations of these group means and our statistical analyses, using Dunnett's multiple comparison test, suggest that decreases in body weight in the 2500-ppm group were slight and not significant when compared with controls (Table 1).

TABLE 5. Effects of INF-6025 on Organ Weights of F_{2b} Rats

	Dietary Level (ppm)			
	0	25	250	2500
			Males	
Brain				
Absolute[a]	1.45±0.06[c]	1.47±0.06	1.50±0.08	1.46±0.10
Relative[b]	2.70±0.14	2.69±0.20	2.83±0.28	3.28±0.24*
Liver				
Absolute	2.17±0.30	2.21±0.29	2.14±0.44	1.84±0.21*
Relative	4.02±0.39	4.04±0.46	3.97±0.38	4.14±0.58
Kidneys				
Absolute	0.67±0.06	0.69±0.06	0.64±0.07	0.50±0.14*
Relative	1.24±0.06	1.27±0.08	1.21±0.09	1.14±0.33
Testes				
Absolute	0.23±0.03	0.24±0.03	0.23±0.03	0.22±0.03
Relative	0.42±0.04	0.44±0.05	0.44±0.03	0.50±0.09*
			Females	
Brain				
Absolute	1.44±0.04	1.41±0.09	1.45±0.06	1.38±0.12
Relative	2.82±0.20	2.85±0.23	3.02±0.37	3.20±0.27*
Liver				
Absolute	2.03±0.25	2.02±0.20	1.89±0.35	1.90±0.26
Relative	3.97±0.34	4.07±0.32	3.86±0.31	4.37±0.34*
Kidneys				
Absolute	0.63±0.02	0.64±0.10	0.63±0.06	0.56±0.05*
Relative	1.24±0.07	1.28±0.19	1.30±0.06	1.29±0.12

[a]Values expressed in grams.

[b]Values represent percent of body weight.

[c]Values represent group means (g) ± SD obtained on day 21.

*Statistically different from control value ($p \leq 0.05$).

Body weight gains for these animals were also slightly less than those reported for controls. Significant reductions in body weights were reported, however, for the 80 males and 80 females exposed to 2500 ppm in the 2-year study with INF-6025 (see Table 2 of the study report). Body weights of F_0 parents in the other dose groups were comparable to controls during the premating period (Table 1). The test material did not appear to have adverse effects on food consumption or food efficiency even at the highest concentration tested (2500 ppm) in this study (Table 2).

No effects on parental mortality, fertility indices (Table 3), or gestation indices (Table 3) were noted for any group.

The viability of F_{1a}, F_{1b}, F_{2a}, and F_{2b} pups was comparable between dose groups and controls (Table 3); however, dietary levels of 2500 ppm were associated with significant reductions in body weights in F_{1a}, F_{1b}, F_{2a}, and F_{2b} pups. Body weights of pups in the other dose groups were comparable to controls (Table 4). Further manifestations of compound-related effects at 2500 ppm were apparent from significant organ weight changes reported at this dietary level (Table 5).

Histopathological findings in F_{2b} pups suggest compound-related effects in cerebellar cytoarchitecture at 2500 ppm; however, our assessment of the biological significance of the "decreased cytoplasmic vesiculation" in livers from pups in the 250- and 2500-ppm groups was precluded by the absence of more specific information. The study author should have indicated if these cytoplasmic inclusions contained glycogen or lipid or if they represented hydropic degeneration. Although these liver findings were reportedly not compound related, the reported information was not sufficient to rule out the possibility of a compound effect.

B. Based on the available data, we cannot rule out the possibility that 250 and 2500 ppm of INF-6025 in the diet had adverse effects in F_{2b} pup livers as demonstrated by statistically significant increases in the histopathological changes discussed above. We recommend that additional information be submitted to further characterize the nature of the reported liver cell inclusions. We further recommend that the testing laboratory use selective stains such as PAS (for glycogen) and Sudan (for lipid) to determine the contents of the cytoplasmic inclusions.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 21-32, and Appendix B, Protocol, CBI pp. 111-152.

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APPENDIX A

Materials and Methods

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Classic Scientific Reviews

Pages 18 through 29 are not included with this copy.
The pages contain detailed methods, protocols, and results
submitted by the pesticide registrant.

APPENDIX B

Protocol

Classic Scientific Reviews

Pages 31 through 70 are not included with this copy.
The pages contain detailed methods, protocols, and results
submitted by the pesticide registrant.

NATIONAL SECURITY INFORMATION (EO 12065)

00479014968

EPA: 68-02-4225
DYNAMAC No. 1-061-A1
March 3, 1986

DATA EVALUATION RECORD

DPX-F6025

Metabolic Study in Rats

STUDY IDENTIFICATION: Hunt, O. R. Metabolism of [phenyl-^{14}C(U)] and [pyrimidine-2-^{14}C] DPX-F6025 by male and female rats. (Unpublished study No. AMR-220-84 performed and submitted by E.I. du Pont de Nemours and Co., Wilmington, DE; 1985.) Accession No. 073802.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-3-86

1. CHEMICAL: DPX-6025; DPX-F6025; ethyl 2-[[[[(4-chloro-6-methoxy-pyrimidin-2-yl)-amino]carbonyl]amino]sulfonyl]benzoate; F-6025; INF-6025; chlorimuron ethyl.

2. TEST MATERIAL: Unlabeled DPX-F6025 had a chemical purity >99 percent; [^{14}C-U-phenyl]DPX-F6025 had a specific activity of 8.4 µCi/mg and a radiochemical purity >99 percent; [^{14}C-2-pyrimidine]DPX-F6025 had a specific activity of 8.2 µCi/mg and a radiochemical purity >99 percent.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Hunt, O. R. Metabolism of [phenyl-^{14}C(U)] and [pyrimidine-2-^{14}C] DPX-F6025 by male and female rats. (Unpublished study No. AMR-220-84 performed and submitted by E.I. du Pont de Nemours and Co., Wilmington, DE; 1985.) Accession No. 073802.

5. REVIEWED BY:

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6. APPROVED BY:

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Signature: Nicholas P. Hajjar
Date: March 3, 1986

Alex Arce, Ph.D.
EPA Reviewer

Signature: _____
Date: _____

Clint Skinner, Ph.D., D.A.B.T.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. The metabolism of [^{14}C]DPX-F6025 was studied in male and female Charles River CD rats. The rats were divided into four groups and dosed with [^{14}C]-labeled DPX-F6025 as follows:

Group 1: A single oral dose of [^{14}C-U-phenyl]DPX-F6025 at 16 mg/kg (low dose).

Group 2: A single oral dose of [^{14}C-2-pyrimidine]DPX-F6025 at 16 mg/kg (low dose).

Group 3: A single oral dose of [^{14}C-U-phenyl]DPX-F6025 at 16 mg/kg (low dose) to rats previously placed on diets containing unlabeled test material at 100 ppm.

Group 4: A single oral dose of [^{14}C-U-phenyl]DPX-F6025 at approximately 3000 mg/kg (high dose).

Excretion of [^{14}C] was apparently independent of sex and dosing regimen, being equally divided between urine and feces (32-53 percent of administered dose). Excretion half-lives for both urine and feces were approximately 50 hours under all dosing conditions. No measurable quantities of radioactivity were observed in expired air. Levels of radioactivity remaining in the tissues of rats 168 hours postadministration were low, accounting for approximately 2-3 percent of the administered dose.

DPX-F6025 was extensively metabolized by both male and female rats. Ten identified and several unidentified metabolites were isolated from the tissues and excreta (see Appendix A for structures.) Conclusions concerning the distribution of metabolites within the tissues, urine, or feces or the effects of sex or dosing regimen on the metabolism of DPX-F6025 could not be made because of deficiencies in the reported data and/or study design.

B. This study is inconclusive, but does provide supplementary data on the metabolism of DPX-F6025.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix B for details.)

1. The animals used in this study were eight male and eight female Charles River CD rats. They were 5-7 weeks old and

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[1]Only items appropriate to this DER have been included.

weighed between 200 and 300 g. The animals were divided into four groups (two rats/sex/group) and dosed as follows:

Group 1: A single oral dose of [^{14}C-U-phenyl]DPX-F6025 at 16 mg/kg (low dose).

Group 2: A single oral dose of [^{14}C-2-pyrimidine]DPX-F6025 at 16 mg/kg (low dose).

Group 3: A single oral dose of [^{14}C-U-phenyl]DPX-F6025 at 16 mg/kg (low dose) to rats previously placed on diets containing unlabeled test material at 100 ppm.

Group 4: A single oral dose of [^{14}C-U-phenyl]DPX-F6025 at approximately 3000 mg/kg (high dose).

For the low doses, 4 mg of the undiluted test material (33.6 µCi of [^{14}C-U-phenyl]DPX-F6025 or 33.2 µCi of [^{14}C-2-pyrimidine]DPX-F6025) was administered to each animal based on a nominal weight of 250 g. For Group 4, 5.0 mg of [^{14}C-U-phenyl]DPX-F6025 (42.0 µCi) was mixed with 750 mg of unlabeled DPX-F6025 and administered to each rat based on a nominal weight of 250 g. The test materials were suspended in ethanol:corn oil (1:9, v/v) and administered by gavage (except for the 100-ppm pretreatment of Group 3).

2. After dosing, all animals were housed individually in metabolism cages equipped with traps for capturing radioactive CO_2. Urine and feces were collected from each rat separately at 6, 24, and 48 hours and on each successive 24-hour period after dosing; the final collection was at sacrifice. Rats from Group 1 were sacrificed at 72 hours postadministration. This time period was originally selected based on the author's experiences with other sulfonyl urea compounds, which had biological half-lives of 12-24 hours. However, due to the longer biological half-life of DPX-F6025, all remaining rats were sacrificed at 168 hours postadministration, except for one male and one female from Group 2, which were sacrificed at 104.5 hours. These two animals, which will be referred to as Group 2A, were sacrificed prematurely in an attempt (apparently successful) to stem a bacterial infection that was spreading in the room. Data from Group 2A are considered by the author to be supplemental. At sacrifice, blood, brain, gastrointestinal (GI) tract, heart, kidney, liver, lung, spleen, testes, ovaries, uterus, fat, muscle, skin, hides, bones, and remaining carcass were taken for analysis. Metabolism cages were thoroughly washed for quantitation of residual [^{14}C].

3. Radioactivity in urine, blood, sodium hydroxide (used to trap any expired $[^{14}C]CO_2$), and the cage washings was quantified by liquid scintillation counting (LSC). Solid samples were freeze dried, pulverized, and combusted, and the released $[^{14}C]CO_2$ was radioassayed by LSC.

4. Preliminary identification and the tissue distribution of DPX-F6052 and its metabolites were accomplished by thin-layer chromatography (TLC). Analyses were performed by cochromatography of samples with known metabolite standards on TLC plates precoated with 0.5 mm of silica gel F-254. Plates were developed using two different solvent systems. Radioactive zones were identified by autoradiography and/or by a TLC linear analyzer. The radiolabel in each zone was quantified by the linear analyzer as well as by scraping the radioactive zones off the TLC plates and counting them in a liquid scintillation counter.

   Metabolites in urine were separated by TLC by spotting raw urine directly onto the TLC plates. Selected urine samples from the Group 3 male rats were also acidified, then treated with β-glucuronidase before TLC analysis to determine conjugated metabolites. Fecal samples were sequentially extracted with n-hexane, methylene chloride:methanol (1:1, v/v), and methylene chloride:methanol:2 M ammonium carbonate (3:4:1, v/v/v). Radioactivity in all three extracts was quantified by LSC, and those that contained sufficient levels of $[^{14}C]$ were further analyzed by TLC as described above (the n-hexane and methylene chloride:methanol extracts were combined for each fecal sample for TLC analysis).

   Organs and tissues containing levels of radioactivity sufficient to be analyzed by TLC were treated as follows. Tissues were extracted sequentially with n-hexane; methylene chloride:methanol:2 M ammonium carbonate (3:4:1, v/v/v); and methanolic hydrochloric acid (1 M). The n-hexane extracts were discarded. The two remaining extracts were concentrated, treated with acetonitrile to remove lipids, and centrifuged, and aliquots of the resulting supernatants were applied to the TLC plates. In tissues where the residues were low, organs were combined (male + male, female + female) for a given group.

5. A more definitive identification of metabolites of DPX-F6025 found in selected samples of urine, feces, and tissues was also made. Extracts of samples containing sufficient radioactivity were streaked onto thicker (1 mm) TLC plates and developed in methylene chloride:methanol:concentrated ammonium hydroxide (150:50:3, v/v/v). Radioactive zones were scraped off and analyzed by high pressure liquid chromatography (HPLC). Two metabolites were also analyzed by mass spectroscopy.

B. Protocol: See Appendix C.

12. REPORTED RESULTS:

A. The actual doses of DPX-F6025 administered to the rats were as follows:

Group 1--3.45 mg, 29.0 µCi; Group 2--3.43 mg, 28.5 µCi; Group 2A--4.06 mg, 33.7 µCi; Group 3--3.90 mg, 32.8 µCi; Group 4--4.35 mg [^{14}C], 36.5 µCi + 650 mg unlabeled, for males and 3.92 mg [^{14}C], 32.9 µCi + 590 mg unlabeled, for females.

B. Data for the excretion and total recovery of radioactivity are presented in Table 1. Recoveries were greater than 90 percent of the administered dose for all groups, except for the female rat in Group 2A. In rats sacrificed 72 hours after dosing (Group 1), 75 percent of the recovered radioactivity was located in the urine and feces. In rats sacrificed at 104.5 hours (Group 2A), 90 percent of the recovered radioactivity was located in the excreta. Greater than 95 percent of the recovered radioactivity was found in the excreta of those animals sacrificed 7 days postadministration.

No radioactivity was detected in the expired air of any group of animals.

All rats maintained for 168 hours postadministration retained less than 2 percent of the administered radioactivity in the organs and tissues, except for the female in Group 2, which retained 3.1 percent (the Group 3 females retained 2.28 percent of the dose, but 0.3 percent was considered to be due to contamination of the hide with excreta). Group 2A rats retained 8-10 percent of the administered dose in the organs and tissues and Group 1 rats, sacrificed at 72 hours postadministration, retained approximately 25 percent.

C. The tissue distribution of [^{14}C]DPX-F6025 residues at the time of sacrifice is presented in Table 2. Measurable levels of [^{14}C] were found in all tissues examined. Concentrations of radioactivity expressed as ppm DPX-F6025 in the organs and tissues for the Group 1 rats ranged from 13.6-20.6 ppm in the livers down to 0.1 ppm in the fat of the male animals. Concentrations of radioactivity in the tissues of Group 2 and 3 rats were much lower than Group 1 rats. Again, the tissue with the highest [^{14}C] concentration was the liver, 1.5 and 1.4 ppm for Group 2 and 3 female rats, respectively. Radioactivity levels in organs and tissues of Group 2A rats were somewhat higher (approximately 5 times) than those in the Group 2 rats, reflecting the shorter maintenance period postadministration. The concentrations of radioactivity measured in the tissues of the Group 4 rats were very low (1 to 35 ppm) when compared to the administered dose of 3000 mg/kg.

TABLE 1. Recovery of Radioactivity From Rats Dosed with [^{14}C]DPX-F6025[a]

Sample	Group 1[b] M	Group 1[b] F	Group 2A[c] M	Group 2A[c] F	Group 2[c] M	Group 2[c] F	Group 3[b] M	Group 3[b] F	Group 4[b] M	Group 4[b] F
Urine										
6-hour	0.1	0.7	0.9	0.6	1.8	2.0	1.2	0.8	0.3	0.5
24-hour	10.6	14.9	12.8	15.7	14.6	9.4	13.8	11.6	5.5	4.1
48-hour	12.7	17.8	10.0	11.9	14.2	12.2	9.8	12.3	27.0	16.8
72-hour	9.9	10.0	6.4	7.0	8.0	9.6	5.6	7.5	14.7	18.5
96-hour	-	-	5.2	4.9	4.3	6.4	3.3	4.1	1.2	5.6
120-hour	-	-	1.3[d]	0.7[d]	2.7	3.3	1.7	2.3	0.4	0.6
144-hour	-	-	-	-	1.3	2.2	1.0	2.0	0.3	0.3
168-hour	-	-	-	-	0.9	1.8	0.5	1.5	0.2	0.1
Total Urine	33.2	43.3	36.6	40.9	47.6	46.9	36.9	42.0	49.4	46.5
Feces										
6-hour	0.02	0.04	NA[e]	0.01	NA	0.02	0.07	0.0	NA	NA
24-hour	3.0	1.1	8.5	8.6	3.8	3.8	9.8	8.6	3.7	2.1
48-hour	16.6	17.3	24.2	8.3	16.5	17.4	21.1	14.5	19.9	7.5
72-hour	14.9	13.9	9.6	9.7	12.6	7.3	10.9	11.6	23.1	22.9
96-hour	-	-	5.2	5.9	6.1	6.3	5.4	6.0	3.1	17.7
120-hour	-	-	1.3	0.5	4.2	4.5	2.7	2.9	0.9	1.9
144-hour	-	-	-	-	1.7	2.8	1.2	1.8	0.6	0.7
168-hour	-	-	-	-	1.0	1.8	0.7	1.5	0.3	0.3
Total Feces	34.4	32.3	48.8	33.1	45.9	43.8	52.8	46.8	51.4	53.1
Organ and Tissues	26.3	25.8	9.4	8.4	1.6	3.1	1.2	2.3	0.3	0.3
Cage Wash	0.7	1.3	0.4	0.8	0.6	0.7	0.4	0.9	0.3	0.2
Total	94.6	102.7	95.2	83.2	95.6	94.5	91.3	91.0	101.5	100.1

[a]All values expressed as percent of administered dose.

[b]Values represent the average of two animals.

[c]Data obtained from a single animal.

[d]Actual time of collection (sacrifice) was at 104.5 hours after dose administration.

[e]NA: not available.

TABLE 2. Distribution of Radioactivity in Tissues (ppm) of Rats Dosed with [^{14}C]DPX-F6025[a]

Tissue	Group 1[b] M	Group 1[b] F	Group 2A[c] M	Group 2A[c] F	Group 2[c] M	Group 2[c] F	Group 3[b] M	Group 3[b] F	Group 4[b] M	Group 4[b] F
Brain	0.3	0.5	0.1	0.1	<0.1	<0.1	<0.1	<0.1	1.0	1.9
Fat	0.1	1.9	0.8	0.5	<0.1	<0.1	<0.1	<0.1	8.8	9.0
GI tract	5.5	5.9	3.0	3.1	0.3	0.6	0.3	0.6	7.4	9.9
Heart	4.7	6.1	1.2	1.0	0.1	0.4	0.1	0.3	2.7	6.8
Kidneys	3.3	6.4	1.5	1.6	0.2	0.7	0.4	0.5	5.1	9.4
Liver	13.6	20.6	6.6	7.2	0.5	1.5	0.8	1.4	7.7	20.5
Lung	4.0	9.7	1.7	2.3	0.1	0.4	0.2	0.4	3.9	8.0
Muscle	1.1	0.6	0.5	0.3	0.1	0.1	0.1	0.1	1.5	3.2
Skin	2.9	2.5	1.3	0.9	0.2	0.3	0.1	0.4	11.5	14.5
Spleen	1.4	2.7	0.5	0.4	<0.1	0.2	0.1	0.2	1.6	4.0
Testes/ ovaries[d]	1.7	9.2	0.8	1.6	0.1	0.5	0.2	0.9	1.7	6.6
Bones	5.5	7.3	1.9	1.2	0.3	0.6	0.3	0.6	10.8	17.9
Carcass	1.5	2.4	0.5	0.4	0.1	0.2	0.1	0.2	2.2	3.6
Hide	5.5	4.8	1.4	0.2	0.2	0.5	0.2	0.4	34.9	17.2
Blood	8.8	12.9	3.3	3.4	0.3	0.9	0.3	0.1	1.6	9.1

[a] All values represent the concentration of [^{14}C]DPX-F6025 equivalents in µg/g of tissue at the time of sacrifice: 72 hours for Group 1, 104.5 hours for Group 2A, and 168 hours for Groups 2-4.

[b] Mean of two rats/sex/group.

[c] Values obtained from a single rat.

[d] Includes uterus also.

Radioactivity found in the hides and GI tracts of rats in all groups were considered by the author to be due to contamination by normal excreta. The biological half-life, the time necessary for the animal to excrete 50 percent of the administered dose, was approximately 50 hours (range, 42 to 58 hours) and appeared to be independent of sex or dose conditions.

D. The distribution of metabolites in the 24-hour feces of rats dosed with [^{14}C-U-phenyl]- or [^{14}C-2-pyrimidine]DPX-F6025 are presented in Table 3. Levels of unmetabolized DPX-F6025 were low, generally comprising less than 10 percent of the radioactivity in the feces. Metabolites detected in the feces of rats dosed with [^{14}C-U-phenyl]DPX-F6025 included (see Appendix A for full names and chemical structures) HOPY-DPX-F6025, HPY-DPX-F6025, ODM-DPX-F6025, DI-HOPY-DPX-F6025, FA-sulfonamide, FA-HOPY-DPX-F6025, sulfonamide, and several unidentified metabolites. The metabolites pyrimidine amine and HOPY-pyrimidine amine were also identified in the feces of Group 2 rats dosed with [^{14}C-2-pyrimidine]DPX-F6025.

The excretion of fecal metabolites with time tended toward increased excretion of more polar metabolites, concomitant with decreased excretion of less polar metabolites as the time post-administration increased. Generally, there were no major sex- or dose-related differences observed regarding excretion or distribution of fecal metabolites of DPX-F6025.

E. The distribution of metabolites in the 24-hour urine samples of rats dosed with [^{14}C]DPX-F6025 are presented in Table 4. For the low-dose groups (Groups 1, 2A, and 3), it was reported that significant levels of unmetabolized DPX-F6025 were excreted in the urine. Metabolites detected in the urine included HPY-DPX-F6025, ODM-DPX-F6025, HOPY-DPX-F6025, FA-HOPY-DPX-F6025, DI-HOPY-DPX-F6025, sulfonamide, FA-sulfonamide, pyrimidine amine, FA-pyrimidine amine, and several unidentified metabolites. The metabolite distribution pattern in (Group 4) urine samples from rats in the high-dose group was considerably different than that from the low-dose group. The less polar compounds, DPX-F6025, HPY-DPX-F6025, and ODM-DPX-F6025, were present at lower levels while the more polar compounds, FA-HOPY-DPX-F6025, DI-HOPY-DPX-F6025, and RU2 showed increased levels.

Treatment of urine of Group 3 male rats with β-glucuronidase did not alter the extractability or distribution of radioactive metabolites compared to untreated urine.

F. Typically, less than 5 percent of the [^{14}C] present in the tissues was extractable with n-hexane, whereas generally more than 75 percent was extractable with methylene chloride:methanol: 2 M ammonium carbonate (3:4:1, v/v/v). An additional small amount was extracted into HCl in methanol, generally 10 percent or less. Total extraction efficiencies were on the order of 80

TABLE 3. Distribution of Metabolites in the 24-Hour Fecal Samples of Rats Dosed with [^{14}C]DPX-F6025

Metabolite	Radioactivity (percent)[a]							
	Group 1		Group 2		Group 3		Group 4	
	M	F	M	F	M	F	M	F
DI-HOPY-DPX-F6025[b]	27.7	17.0	12.7	12.6	7.1	10.9	8.5	12.9
FA-HOPY-DPX-F6025	4.5	4.5	7.9	6.0	11.2	3.1	9.0	7.3
RFU2 at R_f = 0.12[c]	10.3	1.8	14.8	34.3	2.2	7.2	13.3	0.2
FA-sulfonamide	0.	6.1	-	-	3.6	12.3	21.7	32.7
HOPY-DPX-F6025	14.4	23.6	18.7	9.2	22.8	19.3	11.0	17.5
RFU3 at R_f = 0.51[c]	5.3	15.9	3.8	2.8	6.0	6.8	0.2	1.1
ODM-DPX-F6025	2.9	9.1	13.4[d]	26.5[d]	4.1	5.2	7.8	7.4
HPY-DPX-F6025	10.8	10.1	7.8	2.7	7.5	8.6	17.2	7.6
DPX-F6025	9.8	1.1	4.0	1.4	7.2	5.9	5.0	5.1
Pyrimidine amine	-	-	1.6	-	-	-	-	-
Sulfonamide	2.3	1.5	-	-	0.9	4.6	1.2	1.0
Uncharacterized[e]	9.1	4.4	7.7	0.0	0.0	4.0	1.8	3.5
Unextracted	2.9	4.9	7.6	4.5	12.9	12.1	3.2	3.7

[a] Values represent the percent of total fecal radioactivity for each metabolite for the 24-hour collection period. Separation was achieved by TLC using a solvent system of methylene chloride:methanol:concentrated ammonium hydroxide (150:50:3, v/v/v). Metabolites were identified by cochromatography with known standards.

[b] Radioactivity zone was at the origin of the TLC plate. The zone included DI-HOPY-DPX-F6025, but also contained other identified metabolites.

[c] Unknown metabolite at R_f values shown, except Group 2 where R_f values were 0.15 and 0.44 for RFU2 and RFU3, respectively.

[d] These values include metabolite HOPY-pyrimidine amine as well as ODM-DPX-F6025.

[e] Uncharacterized radioactivity was incompletely separated by the TLC system used. Radioactivity may be "tailing of peaks" from identified metabolites or may be small traces of other metabolites too low to be identified.

TABLE 4. Distribution of Metabolites in the 24-Hour Urine Samples of Rats Dosed with [^{14}C]DPX-F6025

Metabolite	Radioactivity (percent)[a]							
	Group 1		Group 2A		Group 3		Group 4	
	M	F	M	F	M	F	M	F
DI-HOPY-DPX-F6025[b]	2.2	3.7	14.7	10.6	19.9	8.2	ND	25.4
FA-HOPY-DPX-F6025	6.7	ND[c]	5.4	4.0	9.5	5.5	42.2	52.7
FA-sulfonamide	0.5	ND	-	-	26.8	38.8	11.8	1.9
PY RU2[d]	-	-	3.8	32.0	-	-	-	-
HOPY-DPX-F6025	27.1	28.0	ND	4.2	6.5	0.1	6.5	8.7
RU2 at R_f = 0.4[e]	-	3.7	-	-	-	-	-	-
ODM-DPX-F6025	22.1	ND	35.0[f]	7.9[f]	16.3	13.9	6.5	2.4
HPY-DPX-F6025	1.4	15.7	1.1[g]	0.8	ND	0.4	6.5	7.0
DPX-F6025	38.9	46.9	40.0[g]	40.6	20.3	32.5	14.5	2.0
Pyrimidine amine	-	-	0.2[g]	0.1	-	-	-	-
Sulfonamide	ND	ND	-	-	0.3	0.9	2.2	ND

[a] Values represent the percent of total urinary radioactivity for each metabolite for the 24-hour collection period. Separation was achieved by TLC using a solvent system of methylene chloride:methanol:concentrated ammonium hydroxide (150:50:3, v/v/v). Metabolites were identified by cochromatography with known standards.

[b] Radioactivity zone was at the origin of the TLC plate. The zone included DI-HOPY-DPX-F6025, but also contained other unidentified metabolites.

[c] None detected, less than 0.1 percent.

[d] Unknown metabolite from the urine of [^{14}C-2-pyrimidine]DPX-F6025-dosed rats.

[e] Unknown urinary metabolite at R_f indicated.

[f] These values also include radioactivity from metabolite HOPY-pyrimidine amine as well as ODM-DPX-F6025.

[g] These values have been placed in their respective categories by our reviewers. They do not agree with the author's placement because of an obvious error in Table XXXIV of the study.

to 99 percent, depending on the sample, except for the kidneys, where up to 54.8 percent of the [^{14}C] was unextractable.

The principal metabolite in the tissue was HPY-DPX-F6025. Other metabolites present in rats dosed with [^{14}C-U-phenyl]DPX-F6025 tissues were ODM-DPX-F6025, HOPY-DPX-F6025, FA-HOPY-DPX-F6025, and sulfonamide. The same metabolites were detected in the tissues of rats dosed with the pyrimidine labeled, except pyrimidine amine, which was observed instead of sulfonamide.

G. Sulfonamide and HPY-DPX-F6025 were identified by mass spectroscopy, cochromatography with two TLC systems, and by HPLC analyses. The remaining metabolites, ODM-DPX-F6025, HOPY-DPX-F6025, FA-HOPY-DPX-F6025, DI-HOPY-DPX-F6025, FA-sulfonamide, pyrimidine amine, and HOPY-pyrimidine amine, were identified by cochromatography in two TLC systems and by HPLC analyses. The unknown metabolites were either present in quantities too low to be identified or could not be identified by the methods employed.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concludes that DPX-F6025 was extensively metabolized by both male and female rats when administered orally at either the low- or high-dose levels. The principal metabolites were HPY-DPX-F6025, HOPY-DPX-F6025, ODM-DPX-F6025, FA-HOPY-DPX-F6025, DI-HOPY-DPX-F6025, FA-sulfonamide, and sulfonamide. Less than 20 percent of the dose remained as DPX-F6025. In general, the same metabolites were observed in excreta and organs and tissues, although the relative amounts varied somewhat.

Excretion of radioactivity in the urine and feces was rapid, with a biological half-life of approximately 50 hours under all dosing conditions. Approximately equal amounts of [^{14}C] were excreted in the urine and feces. The retention of only 2-3 percent of the administered radioactivity at 168 hours postdosing indicates that excretion is the primary route of elimination and that incorporation of DPX-F6025 or its metabolites into tissues is insignificant.

No significant difference in recovery of radioactivity was observed that could be attributed to either sex or dose regimen. Concentration of metabolites varied, but the metabolite distribution was the same for dose regimen and both sexes. The dose levels in this study did not appear to disrupt the metabolic process.

The author proposed pathways for the metabolism of DPX-F6025 in rats (see Appendix E, CBI Figures 22A, 22B, and 22C).

B. A quality assurance statement was signed and dated September 4, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study is inconclusive and provides only supplemental information on the metabolism of DPX-F6025 in rats because of a large number of deficiencies in study design and reported data. Of major importance is the use of only two rats/sex/group instead of the five rats/sex/group that is indicated by EPA guidelines. Furthermore, no individual animal data were presented. Therefore, conclusions of a quantitative nature cannot be made for any aspect of this study.

The author presented no individual animal weights; it is therefore impossible to determine exact doses on a mg/kg basis. Group 1 animals were sacrificed at 72 hours, which was too early (90 percent of the administered dose had not been excreted after 72 hours), and the author dosed only one rat per sex for Group 2 (and 2A), making direct comparisons between dose groups tentative. The information provided to support the author's conclusion that almost the entire oral dose of DPX-F6025 was absorbed was not sufficient to draw that conclusion. Despite these deficiencies, the data on the distribution and excretion of radioactivity following oral administration of [^{14}C]DPX-F6025 were sufficient to lend marginal support for the author's qualitative conclusions concerning those aspects of the study.

Neither quantitative nor qualitative conclusions can be drawn for the distribution of metabolites within the tissues, urine, or feces or for the effects of sex or dosing regimen on the metabolism of DPX-F6025 because of the above deficiencies as well as the following inconsistencies in the reported data. The author reported that large amounts of unmetabolized DPX-F6025 were excreted in the urine (as much as 66.7 percent of urinary metabolites), whereas very little parent compound was excreted in the feces (typically less than 10 percent of fecal metabolites). This is in contrast to what is usually encountered with lipophilic pesticides, yet the author does not discuss this anomaly at all. The author stated (CBI p. 16) that metabolite RUU2 (actually RU2) was increased in the urine of Group 4 rats when compared to Group 1 rats, but no data were presented on this metabolite for Group 4 (see Appendix D, CBI Tables XXXII and XXXIII).

Other inconsistencies were found that raised questions about the reliability of the data or of the data being properly reported. CBI Table XXXIV is improperly labeled. The row for DPX-F6025 was not labeled; instead two rows were designated for ODM-DPX-F6025 and HOPY-pyrimidine amine when in fact (based on Table XXXIII) one row of data should have been labeled ODM-DPX-F6025 + HOPY-pyrimidine amine. Also,

tables presenting data for Group 2 or 2A rats refer to rats/sex/group even though only one animal/sex/group was dosed. This raised questions on whether or not data from the two groups were combined. In CBI Table XXIX, HPY-DPX-F6025 was not detected in the urine of Group 3 male rats on days 1, 2, 3, 4, and 7, but on days 5 and 6 it comprised 65 and 54 percent of the total urinary radioactivity, respectively. Conversely, DPX-F6025, having a very similar R_f value in the TLC system employed, comprised 20-46 percent of the urinary radioactivity on days 1, 2, 3, 4, and 7, but was not detected on days 5 and 6. CBI Table XXXVIII indicates that none of the radioactivity found in the ovaries (+ uterus) of the Group 2 female could be extracted with the solvent systems used, but CBI Table XXXIX shows that 99.3 percent of the radioactivity in the ovaries (+ uterus) of the Group 3 females was extracted using those identical solvents. Finally, CBI Tables XLI and XLII indicate that metabolite HPY-DPX-F6025 was the major metabolite in all tissues examined for Group 1 rats except in the blood, where it was not detected. In the blood of Group 1 rats, as shown in these tables, DPX-F6025 comprised 96.9 and 85.9 percent of the [^{14}C] in males and females, respectively. Parent compound, DPX-F6025, was not detected in the blood of Group 2 or 3 rats, but the metabolite HPY-DPX-F6025 comprised 72 and 69.3 percent of the radioactivity in the blood of Group 2 and 3 females, respectively.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Chemical Structures, CBI Figure 1; Appendix B, Materials and Methods, CBI pp. 2-12; Appendix C, Protocol; Appendix D, Select CBI Tables, CBI Tables XXIX, XXXII-XXXIV, XXXVIII, XXXIX, XLI, and XLII; and Appendix E, Proposed Metabolic Pathways for DPX-F6025, CBI Figures 22A-C.

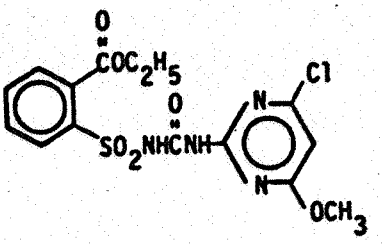
APPENDIX A

Chemical Structures

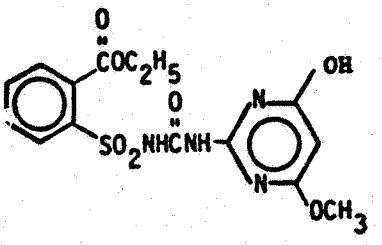
(CBI Figure 1)

FIGURE 1

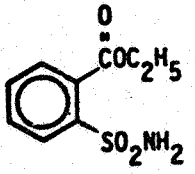
STRUCTURES



DPX-F6025
ethyl 2-[[[[(4-chloro-6-methoxy-2-pyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate

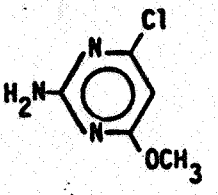


HOPY-DPX-F6025
ethyl 2-[[[[(4-hydroxy-6-methoxy-2-pyrimidin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate

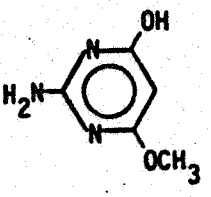


SULFONAMIDE
ethyl 2-[[amino]sulfonyl]benzoate

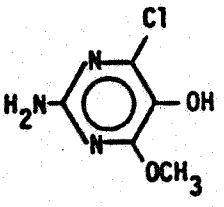
FIGURE 1 (continued)



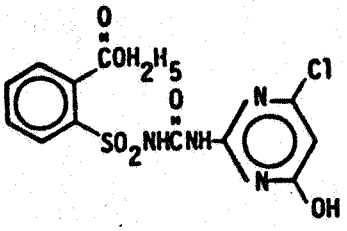
PYRIMIDINE AMINE
4-chloro-6-methoxy-pyrimidin-2-yl amine



HOPY-PYRIMIDINE AMINE
4-hydroxy-6-methoxy-pyrimidin-2-yl amine

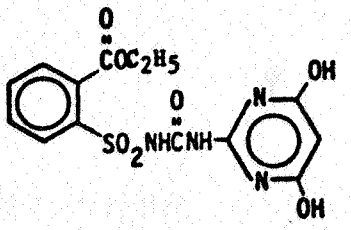


HPY-Pyrimidine Amine
4-chloro-5-hydroxy-6-methoxy-pyrimidin-2-yl amine

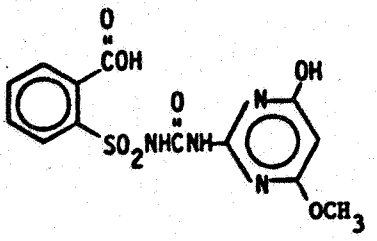


ODM-DPX-F6025
ethyl 2-[[[[(4-chloro-6-hydroxy-pyrimidin-2-yl) amino]carbonyl]amino]sulfonyl]benzoate

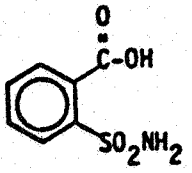
FIGURE 1 (continued)



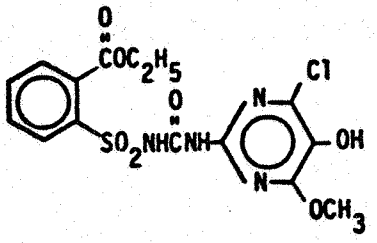
DI-HOPY-DPX-F6025
ethyl 2-[[[[(4-6-dihydroxy-2-pyrimidin-2-yl)
amino]carbonyl]amino]sulfonyl]benzoate



FA-HOPY-DPX-F6025
2-[[[[(4-hydroxy-6-methoxy-2-pyrimidin-2-yl)
amino)carbonyl]amino]sulfonyl]benzoic acid



FA-SULFONAMIDE
2-[(amino)sulfonyl]benzoic acid



HPY-DPX-F6025
ethyl 2-[[[[(4-chloro-5-hydroxy-6-methoxy-pyrimidin-2-yl)
amino]carbonyl]amino]sulfonyl]benzoate

APPENDIX B

Materials and Methods

(CBI pp. 2-12)

Classic Scientific Reviews

Pages 90 through 100 are not included with this copy.
The pages contain detailed methods, protocols, and results
submitted by the pesticide registrant.

APPENDIX C

Protocol

Classic Scientific Reviews

Pages 102 through 126 are not included with this copy.
The pages contain detailed methods, protocols, and results
submitted by the pesticide registrant.

004968

EPA: 68-02-4225
DYNAMAC No. 1-618-1,2a
February 21, 1986

DATA EVALUATION RECORD

DPX-F6025 (INF-6025)

Chronic Feeding Study in Rats

STUDY IDENTIFICATION: Stadler, J. C. Long-term feeding and two-generation four-litter reproduction study in rats with INF-6025. (Unpublished study No. HLR422-85 prepared by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. duPont de Nemours and Co., Wilmington, DE; dated August 26, 1985.) Accession Nos. 073803-073804.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-21-86

1. CHEMICAL: DPX-F6025; INF-6025; F-6025; chlorimuron ethyl; ethyl 2-[[[[(4-chloro-6-methoxy-pyrimidin-2-yl)-amino]carbonyl]amino]sulfonyl]-benzoate.

2. TEST MATERIAL: INF-6025 contained 96 percent active ingredient.

3. STUDY/ACTION TYPE: Chronic feeding study in rats.

4. STUDY IDENTIFICATION: Stadler, J. C. Long-term feeding and two-generation four-litter reproduction study in rats with INF-6025. (Unpublished study No. HLR422-85 prepared by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. duPont de Nemours and Co., Wilmington, DE; dated August 26, 1985.) Accession Nos. 073803-073804.

5. REVIEWED BY:

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Date: Feb. 20, 1986

Robert J. Weir, Ph.D.
Independent Reviewer
Dynamac Corporation

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Date: 2-20-86

6. APPROVED BY:

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Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-20-86

Alex Arce, Ph.D.
EPA Reviewer

Signature: _____
Date: _____

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Under the conditions of the study, INF-6025 was not oncogenic when fed to Sprague-Dawley rats for 2 years at dietary levels of 25, 250, or 2,500 ppm. There were no compound-related signs of toxicity and no effects of dosing on survival. Mean body weights were significantly ($p \leq 0.05$) depressed in high-dose animals; the animals' weights were 8 and 24 percent lower than controls at 104 weeks in males and females receiving 2,500 ppm INF-6025, respectively. There were no compound-related effects of toxicologic importance on clinical laboratory findings, organ weights, gross findings, or nonneoplastic histologic findings. The LOEL based on decreased body weights in males and females is 2,500 ppm and the NOEL is 250 ppm INF-6025.

B. The study is classified Core Minimum for oncogenicity and chronic toxicity; a summary of gross findings was not tabulated.

Item 8--see footnote 1.

9. BACKGROUND: The doses for this study were based on a 90-day feeding study in rats in which there was a lower mean body weight in males receiving 7,500 ppm and females receiving 2,500 or 7,500 ppm INF-6025. There was also a margination of cytoplasmic content of hepatocyte in livers of males receiving 2,500 and 7,500 ppm and of females receiving 7,500 ppm. However, these liver changes were considered adaptive effects.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for complete details.)

Crl:CD(SD)BR rats were housed three/cage, separated according to sex, and acclimated to laboratory conditions for 15 days; during this period the animals were observed for eating habits, toxic signs, and weight gain. Healthy rats were randomized to four groups of 80/sex, housed individually, and fed diets containing 0, 25, 250, or 2,500 ppm INF-6025 active ingredient. Food and water were offered ad libitum.

Diets were prepared weekly, and samples were collected at five intervals for analysis of test compound at each dietary level. Samples were taken at three time intervals to check homogeneity and stability of test compound in the diets.

---

[1] Only items appropriate to this DER have been included.

Animals were observed twice daily for moribundity, mortality, behavior, and appearance, and animals received individual examinations weekly for 6 months and every 2 weeks thereafter. Body weights were determined weekly for 6 months and every 2 weeks thereafter, except a subgroup of rats designated for a reproduction study was not weighed between test days 96 and 225. Food consumption for each group was determined weekly.

Clinical laboratory studies (hematologic, chemical, and urologic) were conducted on 10 rats/sex/group at approximately 3, 6, 9, 12, 18, and 24 months. Hematologic parameters included erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, total and differential leukocytes, and platelets. Slides were prepared for reticulocyte counts, to be examined if needed. Clinical chemistry parameters included alkaline phosphatase, aspartic aminotransferase, alanine aminotransferase activities and serum concentrations of total protein, albumin, urea nitrogen, glucose, cholesterol, creatinine, calcium, sodium, and potassium. Bone marrow smears were prepared from all rats sacrificed at 12 or 24 months, but were only to be read if warranted by experimental results. Urinary parameters examined were volume, pH, urobilinogen, osmolality, glucose, protein, bilirubin, ketone, and occult blood.

Ten animals/sex/group were sacrificed at 12 months, and all survivors were sacrificed at 24 months. Organ weights were determined for brain, heart, liver, spleen, kidneys, and testes. Tissue blocks were prepared for approximately 39 organs/tissues of all animals sacrificed by design, sacrificed moribund, and those that died. A full complement of tissues was examined histologically for males and females fed 0 and 2,500 ppm at the 12- and 24-month sacrifice and for all animals sacrificed in extremis or found dead during the study. For mid- and low-dose groups of sacrificed animals, histologic examination was performed on liver, kidney, lung, and gross lesions.

Samples of brain, liver, kidney, spleen, testis, fat, and blood were collected from 10 rats/sex/group at 12 and 24 months for residue analysis. Samples of urine and feces were also collected, but only at 24 months.

Body weights, weight gains, absolute and relative organ weights, and clinical laboratory data were statistically analyzed by one-way analysis of variance; when there were differences among groups, pairwise comparisons were made using the least significant difference (LSD) test or Dunnett's test. Trend analyses were also performed.

B. Protocol: The Protocol was essentially identical to the Materials and Methods.

12. REPORTED RESULTS:

Dietary Analysis: The mean concentrations of test material in the diet (days 0, 28, 483, 609, and 728) were 26.4±1.3, 264±36, and 2,530±130 ppm for nominal levels of 25, 250, and 2,500 ppm, respectively. All dietary concentrations were within ±20 percent of the nominal levels. Test material was stable in the diets when stored at room temperature for 10 days. Homogeneity of test material in the diets was acceptable; all analytical values except one were 96-106 percent of nominal.

Clinical Observations and Mortality: Clinical signs of toxicity were of a similar incidence in dosed and control groups of males and females, and none were considered dose related. There were no compound-related increases in observable masses. There were no apparent compound-related effects on the incidence of mortality. Survival at week 104 in male groups ranged between 44 and 53 percent; in female groups the range was between 46 and 59 percent (Table 1).

Body Weights and Food Consumption: Table 2 presents mean body weight data at selected intervals during the study. Mean body weights for males receiving 2,500 ppm INF-6025 were significantly lower ($p \leq 0.05$) than controls for the first 12 weeks and at most weighing intervals in the second year of the study and were approximately 8 percent lower than controls at termination. Mean body weights of females receiving 2,500 ppm were significantly lower ($p \leq 0.05$) than controls throughout the study and were approximately 24 percent decreased at study termination. Lower mean weight gains in both high-dose males and females were considered to be related to "dietary intake of INF-6025." Food consumption (0-729 days) in female rats fed 2,500 ppm (18.7 g/rat/day) was lower than in controls (19.8 g/rat/day). Food efficiency was also lower in females receiving 2,500 ppm (0.021 g weight gain/g diet) than in controls (0.029 g weight gain/g diet). Food consumption and food efficiency in males did not differ markedly in any group throughout the study.

Clinical Laboratory Measurements: There were no significant changes in hematologic or clinical chemistry values that were consistent with time or dose; all were within the expected range of biological variations. It was stated that the alterations of red cell counts, hemoglobin, and hematocrit were primarily due to sample hemolysis. Urinary parameters were similar in control and dosed groups.

Organ Weights: At the 12-month sacrifice, the mean absolute and relative spleen weights in males receiving 2,500 ppm were decreased when compared to controls, and the liver-to-body weight ratio in females receiving 2,500 ppm was increased when compared to controls. At the 24-month sacrifice, mean heart weights were decreased in males and females receiving 2,500 ppm and mean kidney weight was decreased in females receiving 2,500 ppm when compared to controls. There were also increases in organ-to-body weight ratios of testes (2,500-ppm group) and liver and brain of females receiving 2,500 ppm (Table 3). These changes in organ-to-body weight ratios were considered to be related to decreased body weights.

TABLE 1. Mortality and Percent Survival in Rats Fed INF-6025 for 2 Years

Dose/Group (ppm)	Mortality and (Percent Survival) at Week			
	26	52	78[a]	104[a]
		Males		
0	2 (97.5)	3 (96.3)	12 (82.9)	39 (44.3)
25	1 (98.8)	3 (96.3)	10 (85.7)	34 (51.4)
250	0(100)	1 (98.8)	8 (88.6)	34 (51.4)
2500	1 (98.8)	2 (97.5)	8 (88.6)	33 (52.9)
		Females		
0	2 (98.8)	3 (96.3)	12 (82.9)	27 (47.1)
25	1 (98.8)	3 (95.0)	10 (85.7)	33 (47.1)
250	2 (97.5)	2 (97.5)	13 (81.4)	38 (45.7)
2500	0(100)	1 (98.8)	9 (87.1)	29 (58.6)

[a]Does not include 10 rats/group/sex sacrificed by design after week 52 (based on 70 rats/sex/group).

TABLE 2. Mean Body Weights (±SD) at Selected Intervals in Rats Fed INF-6025 for 2 Years

Dose/Group (ppm)	Mean Body Weights (g) at Week 0	13	26	52	78	104
			Males			
0	152.4 ±8.05	514.7 ±50.2	600.0 ±69.1	714.7 ±102	768.7 ±111	741.6 ±141
25	149.2* ±7.71	508.6 ±47.3	615.8 ±68.9	711.5 ±115	776.6 ±158	743.9 ±145
250	150.5 ±8.12	506.5 ±47.4	606.3 ±67.8	690.2 ±89.8	726.7 ±137	729.1 ±143
2500	148.3* ±7.41	494.3 ±42.0	591.8 ±64.8	682.5 ±98.1	695.1* ±130	681.8 ±139
			Females			
0	126.0 ±7.57	273.7 ±26.4	314.6 ±39.5	407.7 ±68.2	507.7 ±104	543.2 ±127
25	124.4 ±6.76	270.1 ±23.5	307.2 ±37.2	401.0 ±64.0	478.8 ±92.2	519.7 ±141
250	124.4 ±8.37	272.9 ±30.0	311.3 ±39.4	423.0 ±71.3	515.2 ±101	542.7 ±116
2500	124.7 ±7.54	248.9* ±24.5	277.0* ±27.3	360.5* ±65.7	437.6* ±108	415.8 ±104

*Significantly different from control value ($p < 0.05$).

TABLE 3. Mean Organ Weights and Organ-to-Body Weight Ratios (±SD) in Rats Fed INF-6025 for 2 Years

Sex/Organ	Parameter	Dietary Level (ppm) 0	2,500
		12-Month Sacrifice	
Males			
Spleen	grams	1.025±0.191	0.799±0.152*
	% body weight	0.146±0.030	0.111±0.020*
Females			
Liver	grams	11.742±1.378	12.905±2.002
	% body weight	3.025±0.471	3.591±0.309*
		24-Month Sacrifice	
Males			
Heart	grams	2.190±0.307	1.984±0.313*
	% body weight	0.316±0.089	0.301±0.066
Testes	grams	3.468±1.087	3.857±1.258*
	% body weight	0.493±0.163	0.585±0.201
Females			
Kidney	grams	3.434±0.672	2.987±0.570*
	% body weight	0.654±0.193	0.746±0.195
Liver	grams	16.151±3.459	14.375±3.697
	% body weight	3.003±0.565	3.491±0.464*
Heart	grams	1.566±0.255	1.347±0.204*
	% body weight	0.294±0.059	0.338±0.062
Brain	grams	2.030±0.100	2.073±0.106
	% body weight	0.389±0.088	0.536±0.158*

*Significantly different from control value ($p \leq 0.05$).

Gross Pathology: No compound-related gross effects were observed in animals at 12 months, in animals that died after 12 months, or in animals sacrificed at 24 months. Individual animal data were presented, but there was no summary tabulation of the findings.

Histopathology: At 12 months, there was no increase in any neoplastic lesion in dosed groups when compared to controls. There was an increase in prostatitis in males receiving 2,500 ppm, an increase in fatty replacement in the pancreas in males and females receiving 2,500 ppm, and an increase in portal biliary hyperplasia/fibrosis in females receiving 2,500 ppm (Table 4). These nonneoplastic findings were considered to be chance occurrences or the result of intercurrent disease; because they were commonly occurring changes in rats they were not considered to be related to dosing.

Neoplastic findings for animals that died or were sacrificed moribund in the second year of the study or were sacrificed at study termination are presented in Table 5. There were no increases in dosed males or females. The only nonneoplastic lesion that was increased was portal biliary hyperplasia/fibrosis in males receiving 2,500 ppm (57/68) when compared to control males (44/67). This finding was considered related to aging and not to administration of the test compound.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Under the conditions of the study, INF-6025 was not considered oncogenic when fed to CD(SD)BR rats for 2 years at dietary levels of 25, 250, or 2,500 ppm. Mean body weights in males and females receiving 2,500 ppm were approximately 8 and 24 percent lower than controls at termination; mean body weights in high-dose males were significantly ($p \leq 0.05$) lower than controls during the last 12 months and in high-dose females throughout the study. The decreased weight gain in females receiving 2,500 ppm was accompanied by decreased food consumption and food efficiency. There were no effects of dosing on signs of toxicity or on mortality and no consistent effects on clinical laboratory findings. Several significant differences ($p \leq 0.05$) in organ weights and organ-to-body weight ratios were noted in males and females receiving 2,500 ppm when compared to controls. However, these changes were related to lower body weights and were not accompanied by unusual histologic effects in these organs; hence, they were not considered related to dosing. There were no compound-related gross or histopathologic changes. There was an increased incidence of portal biliary hyperplasia in males that received 2,500 ppm, but the incidence was comparable to that expected for control rats of the Sprague-Dawley strain.

B. A signed list of dated quality assurance audit reports was provided.

TABLE 4. Histopathologic Findings in Rats Fed INF-6025 for 1 Year

	Dietary Level (ppm)							
	Males				Females			
Organ/Lesion	0	25	250	2,500	0	25	250	2,500
Neoplastic								
Reticuloendothelial								
Lymphoma	0/13	1/12	1/12	1/12	0/12	0/13	1/15	0/11
Leukemia	0/13	1/12	0/12	0/12	0/12	0/13	0/15	0/11
Pituitary								
Adenoma	3/13	0/1	0/2	0/11	1/11	3/4	1/6	0/11
Skin/Subcutis								
Fibroadenoma					0/13	0/4	1/6	0/11
Mammary adenocarcinoma					1/13	0/4	1/6	0/11
Adipose tissue								
Lipoma					1/12	0/2	0/2	0/11
Nonneoplastic								
Prostate								
Prostatitis	0/13	0/2	1/2	4/12*				
Pancreas								
Fatty replacement	2/13	0/2	1/2	9/11*	0/12	0/3	0/5	4/11*
Bile duct								
Portal hyperplasia/ fibrosis	8/13	5/12	10/12	5/12	3/12	2/13	4/15	7/11*

*Significantly different from control value ($p \leq 0.05$), Fisher exact one-sided test as analyzed by the author.

TABLE 5. Neoplastic Lesions in Rats Fed INF-6025 for 2 Years[a]

Organ/Neoplasm	Dietary Level (ppm)							
	Males				Females			
	0	25	250	2,500	0	25	250	2,500
Adrenal	(66)[b]	(30)	(32)	(67)	(63)	(35)	(42)	(68)
Adenocarcinoma	0	0	0	0	0	0	2	1
Cortical adenoma	2	1	0	1	4	0	1	1
Pheochromocytoma	18[c]	4	3	12	3	3	0	2
Kidney	(67)	(67)	(68)	(68)	(63)	(64)	(63)	(69)
Lipomatous tumor	1	0	0	2	0	0	1	0
Pancreas	(64)	(28)	(32)	(64)	(61)	(28)	(29)	(68)
Islet cell adenoma	9	6	1	4	5	3	3	2
Pituitary	(67)	(43)	(44)	(67)	(63)	(52)	(52)	(68)
Adenocarcinoma	0	0	0	0	2	3	0	0
Adenoma	42	32	35	44	56	46	47	53
Skin	(67)	(50)	(49)	(68)	(64)	(46)	(51)	(69)
Fibroma	1	3	2	3	0	0	0	0
Cornifying epithelioma	4	3	4	3	0	0	0	0
Lipoma	0	0	1	0	1	0	2	0
Squamous papilloma	5	6	4	5	0	0	1	0
Mammary adenocarcinoma	0	0	1	0	15	9	8	12
Mammary adenoma	0	0	0	1	1	3	0	0
Mammary fibroadenoma	1	0	1	0	18	18	18	10
Thyroid	(62)	(28)	(27)	(65)	(60)	(22)	(31)	(66)
Adenoma, C-cell	6	3	1	4	3	1	1	4
Adenoma, follicular	2	2	1	4	0	1	1	2
Uterus					(62)	(30)	(33)	(69)
Endometrial stromal polyp					3	3	1	1
Leiomyosarcoma					0	0	1	0
Testis	(67)	(40)	(44)	(68)				
Interstitial cell tumor	3	2	2	7				
Mesothelioma	1	2	2	2				
Miscellaneous	(67)	(60)	(51)	(68)	(63)	(34)	(37)	(69)
Histiocytic sarcoma	1	1	1	2	0	0	0	0
Hibernoma, pleural cavity	0	0	0	2	0	2	1	3[c]

[a] Includes animals on test from days 371-742, but not animals that died in the first year or were sacrificed at 12 months. If a neoplasm occurred in only one animal in any group, it was not tabulated.

[b] The numbers in parentheses are the numbers of organs examined.

[c] Includes one malignant neoplasm.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: The design and conduct of the study were adequate except that 20 rats/sex/group were used for a four-litter reproduction study from days 96 to 225. This did not appear to impact on the results of the chronic toxicity/oncogenicity study; clinical laboratory studies did not include this subgroup of animals at 6 months and their body weights were not included in calculating mean values during this period. There was no increase in incidence of any neoplasm and no occurrence of unusual tumors in dosed groups. The highest dose used was the maximum tolerated dose (MTD) based on decreases in mean body weights. We agree with the author's assessment that there were no toxicologically important changes in clinical laboratory findings or on organ weights. A spot check of data on individual animal pathology correlating microscopic findings of nodules and masses with gross observations (Table III of the report) indicated to the reviewers that the correlation was good. There were no summary tables on the incidence of gross pathology and no correlation of nonneoplastic histologic findings with gross findings.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 21-28.

APPENDIX A

Materials and Methods

Classic Scientific Reviews

Pages 140 through 147 are not included with this copy.
The pages contain detailed methods, protocols, and results
submitted by the pesticide registrant.

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EPA: 68-02-4225
DYNAMAC No. 1-061A-2
February 25, 1986

DATA EVALUATION RECORD

INF-6025

One-Year Feeding Study in Dogs

STUDY IDENTIFICATION: Rickard, R. W. and Pastoor, T. P. One-year feeding study in dogs with INF-6025. (Unpublished study No. 232-85 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. duPont de Nemours and Co., Wilmington, DE; dated June 21, 1985.) Accession No. 073802.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-25-86

1. CHEMICAL: DPX-6025; INF-6025; DPX-F 6025-34; benzoic acid, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-ethyl ester.

2. TEST MATERIAL: INF-6025, lot No. 83-233, was of 96.7 percent purity. The appearance of the test material was not described.

3. STUDY/ACTION TYPE: One-year toxicity study in dogs.

4. STUDY IDENTIFICATION: Rickard, R. W. and Pastoor, T. P. One-year feeding study in dogs with INF-6025. (Unpublished study No. 232-85 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for E. I. duPont de Nemours and Co., Wilmington, DE; dated June 21, 1985.) Accession No. 073802.

5. REVIEWED BY:

Robert J. Weir, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Robert J. Weir
Date: 2-24-85

Charles E. Rothwell, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Charles Rothwell
Date: 2-24-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Chronic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-25-86

W. T. Edwards
EPA Reviewer

Signature: _____
Date: _____

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Twenty-four male and 24 female beagle dogs were fed INF-6025 in their diets at 0, 25, 250, or 1500 ppm for 1 year (six dogs/sex/dose). The NOEL for INF-6025 is 250 ppm based on this 1-year dog study. The LOEL is 1500 ppm based on effects on hematology (increased alkaline phosphatase (ALP) in male dogs and decreased erythrocyte (RBC) counts and other effects related to RBC mass and increased leukocyte (WBC) counts in male and female dogs) and increased mean relative liver weight in female dogs.

B. This study is classified Core Guideline.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The stability and concentration of the test material in the diet were measured on test days -1 and 188 and at the end of the study. The diets were also measured for homogeneity of mixing on test days -1, 188, and 204. Samples were collected from the top, middle, and bottom of the mixing vessel for all dietary concentrations.

2. The test animals were male and female beagle dogs obtained from Marshall Research Animals, Inc., North Rose, NY, at approximately 4 months of age. They were acclimated for 4 weeks before treatment was initiated.

3. Twenty-four males and 24 females were randomly distributed into four dose groups consisting of six males and six females each. Each animal was given a daily allotment of Purina Certified Canine Diet No. 5007 (350 g) containing INF-6025 at 0, 25, 250, or 1500 ppm. The dogs were housed individually in stainless steel cages. Identification was by metal collars or tattoo. Tap water was provided ad libitum. Environmental conditions or their control measures were not provided in the report.

4. All dogs were observed for mortality and for abnormal behavior and appearance twice daily. Body weights were recorded weekly (except for week 1), and food consumption was determined on a daily basis. Food efficiency, mean daily diet consumption, and daily intake of INF-6025 were calculated.

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[1] Only items appropriate to this DER have been included.

5. Hematologic [RBC, WBC, differential WBC, and platelet counts; hemoglobin (Hb); hematocrit (HCT); mean corpuscular volume; mean corpuscular Hb content; and mean corpuscular Hb concentration], clinical chemistry [Na^+, K^+, Cl^-, Ca^{++}, glucose, blood urea nitrogen, cholesterol, bilirubin, creatinine, uric acid, total serum proteins, albumin, globulin (calculated), and enzyme activities for ALP, alanine aminotransferase, and asparate aminotransferase], and urinalysis [color, volume, pH, osmolality, protein, glucose, urobilinogen, ketone, bilirubin, occult blood, and microscopic examination of the sediment] determinations were conducted twice prior to dosing[2] and at approximately 1, 3, 6, 9, and 12 months after study initiation.

6. At study termination, all surviving dogs were sacrificed. Necropsies were performed and weights were taken of heart, liver, spleen, kidneys, pituitary, thyroid-parathyroids, adrenals, brain, and gonads. Tissue samples from brain, liver, kidney, spleen, muscle, testes, fat, blood, and urine were taken for determination of tissue retention of the test material. Approximately 43 tissues were taken from each dog for histopathologic examination.

7. Body and organ weight data and clinical laboratory measurements were subjected to analysis of variance. Whenever the ratio of variances indicated significance, the least significant difference or Dunnett's test were employed. The Fisher's exact test was applied to incidences of clinical observations and gross and histopathologic findings. Significance was judged at the $p < 0.05$ probability level.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Dietary Analyses: Dietary analyses indicated that INF-6025 was stable in the diet, the mix was homogeneous, and the overall content of test material in the diet did not vary from the intended concentration by more than 10%.

B. Mortality and Clinical Signs of Toxicity: One female dog showed lethargy and weight loss. It was removed from the study on day 96 and was sacrificed *in extremis* on day 178. Gross and histopathologic examination did not reveal the cause of this dog's weight loss and lethargy. In the female dogs, the incidence of skin sores was significantly ($p < 0.05$) higher in the high-dose group (4) than in the control group (0). In the male dogs, sores were transient and occurred at a comparable rate between dose groups. No other

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[2] These samples are designated pretests 1 and 2 in the report as well as in the tables of this text.

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clinical observations were observed at a rate that differed from the controls or that could be related to the administration of the test substance.

C. Body Weight: The administration of INF-6025 in the diet caused no consistent significant effects on body weight in any dose group when compared to the controls (Table 1).

D. Food Consumption and Food Efficiency: The food consumption for the dosed dogs of both sexes was comparable to that of the appropriate control group. There were, likewise, no significant effects on food efficiency.

E. Hematology: There was a significant decrease ($p < 0.05$) in RBC counts in the high-dose males (Table 2) at the 9- and 12-month intervals and in the mid-dose males at the 3- and 9-month intervals. Significant decreases in RBC were also seen in the females (Table 3) at the 3- and 12-month intervals, and a significant decreasing trend was seen at the 6- and 9-month intervals by regression analysis performed by our reviewers.

Hb was significantly ($p < 0.05$) decreased in the mid-dose males at the 3-month interval. It was always lower in the mid- and high-dose groups than the control group regardless of the sex at all tested intervals. This is considered biologically meaningful though not statistically significant. HCTs were significantly ($p < 0.05$) lower in the high-dose males at the 12-month interval. HCT for the mid- and high-dose groups was always lower, but not statistically significant, than the control regardless of sex. Reticulocyte counts were elevated at pretest and at 3-, 6-, and 12-month intervals for the mid-dose group but not in the high-dose group in either sex. These changes are interpreted as a slight compound-related decrease in RBCs. WBC counts were elevated in the female dogs of the high-dose group and in the male mid-dose group at 6 months.

F. Clinical Chemistry: The results of selected clinical chemistry determinations are presented in Table 4 for the males and Table 5 for the females. ALP determinations were significantly increased ($p < 0.05$) in the high-dose males at 3-, 6-, 9-, and 12-month intervals and in the females at 6 months. Although not statistically significant, an increase that was considered biologically significant was seen at 9 and 12 months in the high-dose females. The additional parameters found to be significant were considered to be either in the range of expected biological variations or unrelated to dosing with INF-6025.

G. Urinalysis: No alterations were found.

H. Organ Weights: At termination, absolute and relative spleen weights were significantly ($p < 0.05$) lower in the high-dose females than in controls. There were no significant differences

TABLE 1. Summary of Mean Body Weights[a] at Selected Intervals for Dogs Fed INF-6025 for 1 Year

Dose Level (ppm)	Week on Study				
	Pretest	2	13	26	39	52
			MALES			
0	7.7 ±0.6		10.7 ±1.5	12.1 ±1.6	12.6 ±1.3	13.3 ±1.5
25	7.7 ±0.4		10.6 ±1.4	11.9 ±1.6	12.8 ±1.9	13.2 ±1.8
250	7.8 ±0.5		9.2 ±1.4	10.4 ±1.1	11.6 ±1.0	11.9 ±0.9
1500	7.8 ±0.4		10.8 ±0.7	11.9 ±1.1	12.3 ±0.8	12.5 ±0.8
			FEMALES			
0	6.9 ±0.7		9.7 ±1.3	10.6 ±1.3	10.8 ±1.6	11.5 ±1.7
25	6.9 ±0.6		10.1 ±0.7	11.2 ±0.8	11.9 ±0.7	12.2 ±1.0
250	7.0 ±0.6		9.8 ±0.7	10.7 ±1.1	11.5 1.1	11.5 1.8
1500	6.9 ±0.5		8.4* ±1.1	9.7[b] ±0.9	10.2[b] ±0.9	10.6[b] ±1.2

*Significantly different from control value ($p < 0.05$).

[a]Values represent the mean ± S.D. body weights (kg) of six dogs.

[b]Only five dogs were used to calculate these values.

TABLE 2. Selected Mean Hematologic Values for Male Dogs Administered INF-6025 for 1 Year

Measurement[a] and Dose (ppm)	Months on Study				
	Pretest	2	3	6	9	12
RBC (x 10^6/μL)						
0	5.72	6.77	6.94	6.97*[a]	7.09*[a]
25	5.96	6.88	7.17	7.17	7.26
250	5.65	6.11*	6.36	6.45*	6.78
1500	5.93	6.38	6.45	6.44*	6.30*
Hb (g/dL)						
0	13.2	16.4	17.0	16.7	17.5
25	13.6	16.5	17.6	17.0	17.9
250	13.2	14.9*	15.7	15.6	16.9
1500	13.7	15.8	16.0	15.8	16.2
HCT (%)						
0	38	46	47	48	50
25	39	46	49	49	51
250	38	42	44	45	48
1500	39	45	45	45	45*
WBC (10^3/μL)						
0	7.8	9.6	10.3	10.0	10.3
25	8.3	10.3	9.3	10.0	8.7
250	7.0	9.1	8.0*	8.9	8.9
1500	7.1	11.6	10.5	9.4	9.5
Reticulocyte (%)						
0	1.01	0.50	0.25	0.52	0.68
25	1.34	0.56	0.37	0.68	1.07
250	1.10	0.41	0.24	0.62	0.75
1500	0.87	0.41	0.20	0.24	0.31

*Significantly different from control value ($p < 0.05$).

*[a]Significant decreasing trend by regression analysis ($p < 0.05$), as calculated by our reviewers.

[a]Abbreviations are as follows: (RBC) red blood cells, (Hb) hemoglobin, (HCT) hematocrit, (WBC) white blood cells.

TABLE 3. Selected Mean Hematologic Values for Female Dogs Administered INF-6025 for 1 Year

Measurement[a] and Dose (ppm)	Months on Study				
	Pretest	2	3	6	9	12
RBC (x 10^6/μL)						
0	5.64	6.98*a	6.61*a	6.99*a	7.10*a
25	5.90	7.45	7.11	7.34	7.15
250	5.54	6.50	6.40	6.47	6.78
1500	5.99	6.16*	6.20	6.39	6.33*
Hb (g/dL)						
0	13.1	17.0	16.2	16.9	17.6
25	13.9	18.3	17.7	18.1	18.4
250	13.1	16.2	16.0	15.8	17.3
1500	14.0	15.4	15.5	15.8	16.2
HCT (%)						
0	37	48	46	49	51
25	40	52	49	51	51
250	37	45	45	46	49
1500	40	44	44	45	46
WBC (10^3/μL)						
0	6.2	9.0	9.0	8.7	8.6
25	7.6	10.9	7.8	9.6	10.2
250	6.8	11.3	10.5	10.3	10.5
1500	8.6*	13.6*	14.0*	12.8*	14.2*
Reticulocyte (%)						
0	0.53	0.28	0.09	0.23	0.29
25	0.83	0.63	0.26	0.48	0.72
250	1.37*	0.83*	0.76*	0.80	1.97*
1500	0.81	0.44	0.40	0.29	0.42

*Significantly different from control value ($p < 0.05$).

*[a]Significant decreases by regression analysis ($p<0.05$) as calculated by our reviewers.

[a]Abbreviations are as follows: (RBC) red blood cells, (Hb) hemoglobin, (HCT) hematocrit, (WBC) white blood cells.

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TABLE 4. Summary of Selected Mean Clinical Chemistry Data[a] for Dogs Fed INF-6025 for 1 Year

Dose Level (ppm)	ALP[b] (U/L)	Bilirubin (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)	Na^+ (mmol/L)	Cl^- (mmol/L)
			MALES, Pretest 2			
0	104.7 ±8.1	0.187 ±0.053	0.48 ±0.20	0.673 ±0.046	148.3 ±1.2	109.8 ±1.2
25	96.3 ±17.1	0.202 ±0.023	0.43 ±0.15	0.698 ±0.097	148.7 ±2.5	108.3* ±0.8
250	92.0 ±11.7	0.177 ±0.020	0.48 ±0.12	0.692 ±0.067	148.3 ±0.8	108.7 ±0.8
1500	102.0 ±18.0	0.222 ±0.066	0.42 ±0.23	0.668 ±0.043	147.7 ±1.4	108.0* ±1.1
			MALES, 6 Months			
0	39.2 ±7.9	0.217 ±0.021	0.38 ±0.29	0.858 ±0.061	149.5 ±1.5	113.2 ±1.3
25	47.2 ±5.1	0.250 ±0.067	0.50 ±0.25	0.870 ±0.064	150.3 ±2.7	112.8 ±2.6
250	58.5 ±10.7	0.182 ±0.024	0.035 ±0.26	0.810 ±0.118	148.5 ±1.8	112.5 ±1.0
1500	111.8* ±49.4	0.178 ±0.048	0.25 ±0.15	0.857 ±0.078	150.2 ±1.0	112.5 ±2.4
			MALES, 12 Month			
0	39.5 ±6.9	0.297 ±0.032	0.22 ±0.26	0.838 ±0.081	149.0 ±2.2	116.0 ±1.7
25	43.8 ±15.8	0.215* ±0.024	0.67* ±0.33	0.810 ±0.068	148.8 ±1.0	115.3 ±1.2
250	64.3 ±21.1	0.255 ±0.033	0.12 ±0.15	0.830 ±0.121	148.7 ±2.0	115.2 ±1.5
1500	111.8* ±45.8	0.142* ±0.024	0.37 ±0.28	0.798 ±0.091	150.8 ±1.5	115.5 ±1.8

*Significantly different from control value ($p < 0.05$).

[a]Values represent mean ± S.D. from six dogs.

[b]Alkaline phosphatase.

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TABLE 5. Summary of Selected Mean Clinical Chemistry Data[a] for Dogs Fed INF-6025 in 1 Year

Dose Level (ppm)	ALP[b] (U/L)	Bilirubin (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)	Na^+ (mmol/L)	Cl^- (mmol/L)
			FEMALES, Pretest 2			
0	135.0 ±48.1	0.133 ±0.068	0.38 ±0.10	0.570 ±0.060	148.7 ±0.8	109.7 ±1.8
25	137.2 ±50.3	0.108 ±0.035	0.40 ±0.14	0.573 ±0.049	148.7 ±0.5	108.0 ±2.1
250	96.3 ±25.2	0.173 ±0.085	0.43 ±0.08	0.562 ±0.042	149.8 ±2.6	109.8 ±2.4
1500	87.7 ±17.1	0.120 ±0.070	0.33 ±0.19	0.555 ±0.043	149.0 ±1.1	110.0 ±1.1
			FEMALES, 6 Months			
0	59.2 ±34.3	0.212 ±0.035	0.32 ±0.20	0.783 ±0.094	152.0 ±1.3	114.0 ±2.0
25	41.3 ±19.3	0.237 ±0.056	0.15 ±0.05	0.808 ±0.051	151.8 ±1.7	113.7 ±1.0
250	49.3 ±30.1	0.238 ±0.049	0.32 ±0.24	0.807 ±0.091	149.7 ±1.6	114.7 ±1.4
1500	131.8* ±73.5	0.198 ±0.022	0.24 ±0.09	0.752 ±0.082	151.6 ±2.3	117.2 ±4.5
			FEMALES, 12 Months			
0	70.8 ±46.9	0.348 ±0.059	0.57 ±0.12	0.812 ±0.103	151.8 ±2.4	117.3 ±3.0
25	50.3 ±26.1	0.292 ±0.066	0.57 ±0.14	0.948* ±0.093	152.3 ±2.3	117.2 ±2.1
250	63.5 ±32.4	0.238* ±0.042	0.43 ±0.18	0.852 ±0.074	150.7* ±1.2	116.2 ±2.7
1500[c]	135.6 ±75.3	0.186* ±0.030	0.26* ±0.13	0.808 ±0.061	151.8 ±2.3	116.8 ±1.3

*Significantly different from control ($p < 0.05$).

[a]Values represent mean ± S.D. from six dogs.

[b]Alkaline phosphatase.

[b]Only five dogs were available to generate these values.

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in any other absolute organ weights in either sex. The relative testes weights for mid-dose males were significantly different ($p < 0.05$) from the controls but no biological meaning was attached to this finding since it was not dose related. The relative liver weight was significantly ($p < 0.05$) different from the controls in the high-dose females.

I. Gross and Histopathologic Pathology: No compound-related gross or microscopic lesions were noted. Congestion in the spleen was noted in all groups and was considered to have resulted from the method of sacrifice.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "The overall no-observable-effect level (NOEL) for this study was 250 ppm based on compound-related effects in the high-dose groups (1500 ppm) that included hematological effects in male and female dogs, increases in the mean serum alkaline phosphatase activity in male dogs, and an increase in the mean relative liver weight in female dogs."

B. A signed, undated quality assurance statement was presented in the report; however, the report was signed and dated (June 20, 1985) by an auditor from the Quality Assurance Section.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. The study was conducted with homogeneously mixed diets. The test substance was stable for the period between mixes and the overall test substance content of the diets was within 10% variation of the nominal concentration. The dogs in all test groups gained body weight at a rate similar to the controls despite inconsistencies in the first 3 months. Food consumption and food efficiency of the test groups were comparable to the respective controls. Although the number of sores on the skin of high-dose male and female dogs was higher than those in the controls, this was not considered by either the authors or reviewers to be directly compound induced but rather due to rubbing against the cage. Whether this could have been a secondary effect of some compound induced itching or other immeasurable behavioral effect cannot be determined. No other meaningful clinical observations were made. One high-dose female dog was sacrificed *in extremis* on day 178 of the study after having been removed from the study on day 96 due to weight loss and lethargy. Gross and histopathologic examination of the dog's tissues did not reveal the cause of the symptomatology.

Mortality was not affected by the test substance. Compound-related decreases were seen in RBC counts, HCT values, and Hb concentrations. The occasional changes in RBC mass seen in the 250-ppm group were considered to be more a function of the small sample size than a compound-related effect since RBC counts, HCT, Hb, and reticulocyte values did not portray a consistent trend. The effects were small and not accompanied by hemosiderosis. ALP activity was significantly elevated in the high-dose males at 3, 6, 9 and 12 months and in the high-dose females at 6 months. The high-dose females showed elevated WBC counts due to increased neutrophils and monocytes. The above hematologic effects and the increase in mean ALP activity were considered to be due to the test material. The high-dose females showed significantly reduced absolute and relative spleen weights, which were considered to be due to sacrifice method. The increased relative liver weight for the female high-dose group was considered compound related by the reviewers primarily because of the increased ALP activities even in the absence of alterations in liver morphology. The significantly increased testes weights in the mid-dose male group is not considered compound related as it was not dose related and there were no correlated morphologic changes. Splenic congestion in all groups was considered to be induced by the method of sacrifice. No other morphologic effects, gross or microscopic, were noted.

B. The reviewers agree with the study authors that the NOEL is 250 ppm and the LOEL is 1500 ppm based on RBC- and WBC-related changes, increased ALP activity, and increased relative liver weight.

C. There were no problems, discrepancies, or inaccuracies in the design, conduct, or reporting of the study that would have an adverse effect on the validity of the study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 60-82.

APPENDIX A

Protocol

Classic Scientific Reviews

Pages 161 through 183 are not included with this copy.
The pages contain detailed methods, protocols, and results
submitted by the pesticide registrant.