KOB-1448
TXR-5924

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

005924

JUN - 4 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Developmental Toxicity (teratology) Study with FOLPAN in Rats (Makhteshim Study No. MAK/049/FOL). EPA Identification No. 11678-18; Caswell No. 464; Toxicology Branch Project No. 1114.

FROM: Alan C. Levy, Ph.D.
Toxicologist, Review Section V
Toxicology Branch/HED (TS-769C)
Alan C. Levy 6/3/87

TO: Ms. Lois Rossi (PM 21)
Registration Division (TS-767 C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V
Quang Bui 6/3/87

and

Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Makhteshim-Agan (America) Inc.

Action Requested: Review a Developmental Toxicity (teratology) Study with FOLPAN.

Recommendations: The Maternal No Observed Effect Level (NOEL) is 150 mg/kg/day. The Maternal Lowest Observed Effect Level (LOEL) is 550 mg/kg/day. There were reductions in food consumption and body weight gain in the mid-dose group (550 mg/kg/day). In addition, 2/22 rats in this group had soft feces compared to none in control.

The Developmental Toxicity No Observed Effect Level (NOEL) cannot be determined. The Developmental Toxicity Lowest Observed Effect Level (LOEL) is 150 mg/kg/day (LDT). There was a trend increase in "small" fetuses with a non-significant difference from controls in the low-dose (150 mg/kg/day) and statistical significance in the mid- and high-dose groups (550 and 2000 mg/kg/day). A statistically significant increase in reduced ossification of interparietal bone as well as an increase in angulated ribs was observed in low-dose fetuses. Two fetuses with malformations occurred in the

high-dose group: one with unilateral microphthalmia and one with no tail, anus imperforate, body constricted at level of diaphragm, posterior portion of axial skeleton appeared to be missing, testes ectopic (medial to kidneys), left kidney misshapen, adrenals pale and ectopic (anterior and medial), ureters absent.

The developmental toxicity study with FOLPAN in rats (Life Sciences Research Israel Ltd./Makhteshim-Agan Study No. MAK/049/FOL) is classified as Core-Supplementary Data. A new study is required.

The results of this study indicate that, at least in rats, FOLPAN is a potential developmental toxicant: adverse effects on the developing fetus were observed at doses lower than those producing maternal toxicity.

Complete evaluation of the developmental toxicity potential of FOLPAN must await the submission of a new study with dosage levels lower than 150 mg/kg/day.

A developmental toxicity risk assessment could not be performed at the present time in the absence of a developmental toxicity NOEL from an acceptable study and exposure levels generated by the Exposure Assessment Branch.

Primary Reviewer: Alan C. Levy, Ph.D.
Review Section V/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head
Review Section V/HED (TS-769C)

I. Study Type: Teratology
(Guideline § 83-3)

Study Title: FOLPAN - Teratology Study in the Rat

EPA Identification Numbers:

EPA Identification: 11678-18
EPA Accession: 260781
EPA Record: 168096
Shaughnessy: 081701-5
Caswell: 464
Tox. Branch Project: 1114
Document:

Sponsor: Makhteshim-Agan (America) Inc.
Two Park Avenue
New York, NY 10016

Testing Laboratory: Life Sciences Research Israel Ltd.
PO Box 139
Ness Ziona, Israel.

Study Number: Life Sciences Research Israel Ltd. No.
MAK/049/FOL

Study Date: November 10, 1985

Study Authors: Drs. Y. Rubin and A. Nyska

Test Material:

Trade Name: FOLPAN
Common Name: Folpet
Chemical Name: N-(trichloromethylthylthio)-phthalimide
Grade: Technical
CAS Number: 133-07-3
Molecular Weight: 296.58 (pure substance)
Appearance: White powder
Stability: Incompatible with alkalis
Melting Point: 177°C (pure substance)
Purity: 91.1%
Typical Impurities: [redacted]

005924

Vehicle: 0.5% carboxymethyl cellulose (CMC) prepared in 0.5% acetic acid.

Test Animal: Nulliparous female CD rats obtained from Charles River Breeding Laboratories (UK) Ltd. Stock males were from the same strain and source.

This study was designed to investigate the developmental toxicity potential of FOLPAN.

II. Material and Methods: A copy of the methods section from the investigators' report is appended.

A. Analytical Results

It was indicated that the batch identity, purity and stability of the test material were the responsibility of the Sponsor. The test material was formulated on the day of use. Achieved concentrations and short term stability of FOLPAN in the doses were measured once during the course of the study:

Group	Dosage (mg/kg/day)	volume-dosage (ml/kg/day)	Expected Concentration (mg/ml)	Analysis (mg/ml) Time 0	3.5h
1	0	10	0.0	ND(a)	ND(a)
2	150	10	15.0	17.1 ±0.8(c)	14.0 ±0.4(b)
3	550	10	55.0	49.0 ±1.7(c)	46.6 ±1.4(c)
4	2000	10	200.0	208.5 ±14.3(c)	206.7 40.4(c)

ND:None detected Appendix 5, p.48 of report
(a)-1 determination (b)-2 determinations (c)-3 determinations

Appendix 5, p. 48: achieved concentration and stability of FOLPAN in formulated doses - The analyses for Groups 2, 3 and 4 indicate a mean of two or three determinations ± a value. Since there is no indication that this is S.D. (standard deviation) or S.E. (standard error), the assumption is made that the ± value represents a "range". The ± values at time 0 are 5, 3 and 7% of means for Groups 2, 3 and 4, respectively. The ± values at 3.5 hours are 0, 3 and 20% for Groups 2, 3 and 4, respectively. It appears, therefore, that at the 3.5 hour Group 4 determinations, the analysis values ranged from 166.3 to 247.1 mg/ml (mean of 206.7). Please comment on the relatively large range in Group 4 in relation to the actual amount of FOLPAN received by the animals as well as the ± values (S.D., S.E. or range).

005924

Statistical methodology was described in detail.

A quality assurance Statement was included.

III. Results

A. Gross maternal observations: Of 21 high-dose animals (2000 mg/kg/day), 21 had soft feces, 4 had staining of body fur and 8 had perianal staining. Two mid-dose rats (550 mg/kg/day) had soft feces.

The above findings in the high-dose animals are considered to be compound related, with soft feces in the mid-dose group being a possible effect.

One high-dose rat (no. 83) was found dead on day 16 of gestation. It was stated in the report that post-mortem examination showed that death was caused by multiple hemorrhagic ulcerations of the gastric mucosa.

The report states that high-dose rat no. 85 was found to be "not pregnant" at necropsy on day 20 (p. 11, 5.4 Terminal Findings). This animal was also noted as "not pregnant" on p. 72. However, on p. 47, the report states that the animal was pregnant. Please comment.

B. Body weight during gestation (g) - group mean values and standard deviations

Group No.	Dose (mg/kg/day)	Day Post Coitum 0	6	15	20	B.W. Change Days 6-15[e]
1	0	227.0[a] ±7.4[b]	255.8 ±9.1	303.0 ±14.0	380.7 ±21.2	47.3 ±9.3
2	150	230.3 ±7.0	259.8 ±9.1	309.2 ±14.8	381.9 ±22.1	49.0 ±9.9
3	550	229.0 ±6.6	254.5 ±8.2	293.4 ±11.2	356.3[cd] ±21.3	38.9[d] ±6.1
4	2000	228.4 ±7.1	254.2 ±9.4	272.9[f] ±28.4	344.3[d] ±17.5	18.8[d] ±22.7

a = Mean
b = Standard Deviation (S.D.)
c = Reviewer's calculation, report states 156.3
d = Significantly different from control, $p < 0.001$, Student's "t" test
e = Reviewer's calculations; values obtained from individual animals
f = Mean does not include no. 83 which died day 16 (report mean of 268.7 includes no. 83)

Data extracted from the report: Table 3, p. 20, Study MAK/049/FOL

005934

Responses requested:

P.19 - Table 2, group 3, gestation days 1-20: the value of 26.7 should be 26.8.

P.55 - Appendix 7 (continued), dam no. 64, gestation days 17-20: is the value 55.3 correct? The next highest value in the group is 37.3 and the values for dam No. 64 during days 14-16 appear to be similar to those of other dams. Please comment.

C. Cesarean Section Observations

Group mean litter data at Day 20 of gestation - group mean values and standard deviations (From the report: Table 4, p. 21, Study MAK/049/FOL)

Group mean litter data at Day 20 of gestation - group mean values and standard deviations

Group	: 1	2	3	4
Test material	: Control	-	FOLPAN	-
Dosage (mg/kg/day)	: 0	150	550	2000

GRP		Corpora lutea	Weight of gravid uterus (g)	Live fetuses M	F	T	Resorptions E	L	T	Implantation loss* Pre- %	Post- %	Mean fetal weight (g)	Mean CRL (mm)	Mean placental weight (g)
1	i	17.6	81.9	8.2	7.1	15.3	0.6	0.0	0.6	8.8	4.7	3.50	35.1	0.47
	ii	3.1	9.8	2.0	2.4	2.4	0.8	0.0	0.8	2.1	1.2	0.21	0.9	0.04
	iii											0.23	1.2	0.05
2	i	16.7	77.7	7.7	6.7	14.4	0.9	0.1	1.0	8.8	5.7	3.47	35.2	0.46
	ii	2.4	14.7	2.9	1.9	2.7	1.8	0.3	1.8	1.7	3.0	0.21	1.2	0.05
	iii											0.20	1.0	0.06
3	i	16.0	65.0[c]	6.2	6.2	12.5[b]	1.3	0.0	1.3	12.3[b]	10.1[c]	3.37	34.6	0.49
	ii	2.9	17.8	2.5	2.3	3.6	1.5	0.2	1.5	6.6	2.5	0.32	1.3	0.13
	iii											0.25	1.4	0.06
4	i	16.3	68.5[b]	7.4	6.5	13.9	0.7	0.0	0.7	9.9	5.1	3.24[b]	34.1	0.42
	ii	2.4	13.2	2.5	2.9	3.0	1.0	0.0	1.0	4.4	1.7	0.32	1.9	0.08
	iii											0.23	1.2	0.05

* Freeman-Tukey transformed data

i - group mean value
ii - standard deviation
iii - pooled within-litter standard deviation

M = male; F = female
E = early; L = late; T = total
CRL = crown rump length

b: significantly different from controls, $p < 0.01$, Student's 't'-test
c: significantly different from controls, $p < 0.001$, Student's 't'-test

005924

Responses requested:

P.66 - Appendix 9 (continued), dam no. 21, Mean CRL: the value of 36.5 should be 36.1 (P. 89, total of 433 CRL, divided by 12 is a mean of 36.08 or 36.1).

P.67 - Appendix 9 (continued), dam no. 25, mean fetal weight: the value of 3.49 should be 3.51 (P.92, total of 45.59 fetal weight, divided by 13 is a mean of 3.5069 or 3.51).

P.67 - Appendix 9 (continued), dam no. 28, Mean CRL: the value 36.3 should be 36.1 (P. 93, total of 253 CRL, divided by 7 is a mean of 36.143 or 36.1).

P.67 - Appendix 9 (continued), dam no. 35, pre-implantation loss: the value of 11.8 should be 17.6 (13 live fetuses, 1 late resorption, 17 corpora lutea -17 17 minus 14 is 3, 3 divided by 17 is 17.6%).

P.67 - Appendix 9 (continued), dam no.35, post-implantation loss: the value of 6.7% should be 7.1% (13 Live fetuses, 1 late resorption - 13 + 1 is 14, 1 divided by 14 is 7.143%).

P.69 - Appendix 9 (continued), dam no. 56, Mean CRL: the value of 33.7 should be 34.0 (P.108, total of 510 CRL, divided by 15 is a mean of 34.00 or 34.0).

P.71 - Appendix 9 (continued), dam no. 76, Mean fetal weight: the value of 3.35 should be 3.34 (P.119, total of 43.43 fetal weight, divided by 13 is a mean of 3.3408 or 3.34).

Statistically Significant Fetal Observations at Necropsy - Small fetus (< 3.0 g or > 0.5 g below litter mean)

Dose (mg/kg/day)	0	150	550	2000
No. Fetuses Examined	336	316	274	277
No. Litters Examined	22	22	22	20
no. fetuses/ no. litters	20/9	22/9	33/10	75/15
% fetuses/% litters	5.95/ 5.75	6.96/ 7.29	12.04[a]/ 12.49[c]	27.08[b]/ 24.60[d]

a = significantly different from controls, $p < 0.05$, Chi-square test
b = significantly different from controls, $p < 0.001$, Chi-square test
c = significantly different from controls, $p < 0.05$, Student's "t" test applied to Freeman-Tukey transformed data
d = significantly different from controls, $p < 0.001$, Student's "t" test applied to Freeman-Tukey transformed data

Data extracted from the report: Table 6, p.23 and 24, Study MAK/049/FOL

The mid-dose group (550 mg/kg/day) had mean values indicating less live fetuses and more pre- and post-implantation loss. As the high dose values for these parameters were similar to controls, the mid-dose results do not appear to be caused by compound administration. The decrease in mean fetal weight of the high-dose group (2000 mg/kg/day) indicates the possible effect of FOLPAN on fetal growth.

Two high-dose fetuses (dam no. 75) showed malformations:

Fetus # 1. External: No tail, anus imperforate, body constricted at level of diaphragm, posterior portion of axial skeleton appeared to be missing.

Internal: Testes ectopic (medial to kidneys), left kidney misshapen, adrenals pale and ectopic (anterior and medial), ureters absent.

Fetus # 2. Marked unilateral microphthalmia

Responses requested:

P. 130 - Appendix 11 (continued), dam no. 58, External Observation, small fetus (< 3.0 g or > 0.5 g below litter mean): the value of 9/14 (9 small fetuses out of 14 total fetuses) should not be present (value is 0/14, see P. 109). Error probably due to typing of value for dam no. 57 also under dam no. 58.

P. 130 - Appendix 11 (continued), dam no. 58, Internal Observation, Petechial hemorrhages on surface of lung and Ureter distended unilaterally or bilaterally: the indication of one fetus with ureter distended should be for dam no. 59 and not for no. 58 (see P. 109).

D. Free-hand skeletal sections: The only parameter that showed statistical significance from the control value was "discoloration/pallor of liver" in the high-dose group (9/139, 6.5%; control - 2/164, 1.2%). In addition, one high-dose fetus had "marked unilateral microphthalmia."

E. Skeletal Examinations-

1. Observation skeletal examination number (percent) of affected fetuses (Data extracted from Table 9, p.29-32, Study MAK/049/FOL)

Group Dose (mg/kg/day) No. fetuses examined	1 0 171	2 150 160	3 550 138	4 2,000 134
SKULL				
Anterior fontanelle large	18(10.53)	19(11.88)	35(25.36)[b]	54(40.30)[c]
Reduced ossification of supraoccipital bone	25(14.62)	29(18.13)	28(20.29)	40(29.85)[b]
Reduced ossification of intraparietal bone	23(13.45)	29(18.13)	22(15.94)	49(36.57)[c]
Reduced ossification of parietal bone(s)	5(2.92)	10(6.25)	6(4.35)	15(11.19)[b]
Reduced ossification of squamosal bone(s)	4(2.34)	5(3.13)	11(7.97)[a]	11(8.21)[a]
SPINAL COLUMN AND THORAX				
Angulated ribs	0(0.00)	5(3.13)	4(2.90)	6(4.48)[a]
One or more sternebrae 1-4 unossified	1(0.58)	3(1.88)	5(3.62)	14(10.45)[c]
APPENDICULAR SKELETON				
Reduced ossification pubic bone(s)	12(7.02)	11(6.88)	11(7.97)	22(16.42)[a]
Metatarsal v unossified bilaterally	6(3.57)	8(5.10)	16(12.12)[a]	11(8.59)

NOTE: Parameters listed are only those which had a statistically significant difference.

Significantly different from control by chi-square test:
a = $p < 0.05$ b = $p < 0.01$ c = $p < 0.001$

005924

2. Observations at skeletal examination - litter distribution of affected fetuses (Data extracted from Table 10, P. 33-36, Study MAK/049/FOL)

Group Dose (mg/kg/day) No. litters examined	1 0 22	2 150 22	3 550 22	4 2,000 20
	N(mean %)	N (mean %)	N(mean %)	N(mean %)
SKULL				
Anterior fontanelle large	10(10.77)	9(11.42)	14(28.99)[c]	15(37.02)[c]
Reduced ossification of supraccipital bone	10(13.92)	13(17.76)	10(22.68)[b]	16(32.52)[c]
Reduced ossification of intraparietal bone	12(13.23)	13(18.73)[a]	12(18.56)[a]	19(38.53)[c]
Reduced ossification of parietal bone(s)	3(2.59)	5(5.76)	4(9.52)[b]	11(10.52)[c]
Reduced ossification of squamosal bone(s)	4(2.16)	4(2.84)	6(7.66)	9(8.31)[c]
SPINAL COLUMN AND THORAX				
Angulated ribs	0(0.00)	3(2.84)[b]	3(6.49)[c]	5(6.51)[c]
One or more sternebrae 1-4 unossified	1(0.57)	2(1.87)	5(4.22)[c]	9(11.27)[c]
APPENDICULAR SKELETON				
Reduced ossification of pubic bone(s)	8(6.81)	7(6.74)	10(8.14)[a]	11(16.94)[c]
Fewer than 3 metacarpal bones unossified on one or both manus	4(2.57)	4(4.92)	7(5.63)[b]	6(10.39)[c]
Metatarsal V unossified bilaterally	4(3.19)	5(6.60)	9(12.62)[c]	6(9.17)[c]

N = number of litters with one or more affected fetuses

$$\text{Mean \%} = \frac{\text{Sum of individual litter frequencies}}{\text{Number of litters}} \times 100$$

Significantly different from control by Student's "t" test applied to Freeman-Tukey transformed data.

The statistically significant differences noted in the low-dose group were an increase in angulated ribs when the number of litters with this observation were compared with controls (no difference when numbers of total pups were compared) and an increase in the percent of litters containing fetuses with reduced ossification of intraparietal bone. Numerous skull, spinal column/thorax and appendicular skeletal differences were reported between mid- and/or high-dose fetuses/litters and control values. These results indicate that all three doses of FOLPAN appear to have an effect on skeletal development.

IV. Conclusions

The Maternal No Observed Effect Level (NOEL) is 150 mg/kg/day. The Maternal Lowest Effect Level (LOEL) is 550 mg/kg/day. There was a decrease in food consumption in the mid-dose group only during days 7-9 and in the high-dose group during days 7-9, 10-13, 7-16 and 1-20. The high-dose rats ate more than the controls during days 17-20 (post compound administration). Body weight was below control mean (statistically significant) in the mid-dose group on days 17 and 20 and in the high-dose rats on days 8-20. Body-weight gain was less than control for mid- and high-dose groups during days 0-20 and 6-20. High-dose animals were observed to have soft feces and stained fur. Post-implantation loss occurred more frequently in the mid-dose group (550 mg/kg/day) than in the control. As there was essentially no difference between the high dose and the control, it is questionable whether the mid-dose observation is treatment related.

The Developmental Toxicity NOEL cannot be determined from the available data. The Developmental Toxicity LOEL is 150 mg/kg/day. Mid- and high dose groups had fetal weights less than control with larger numbers of small fetuses (< 3.0g or > 0.5g below litter mean). Fetal length was decreased in the high-dose rats (slight decrease in the mid dose). There was a slight increase in the frequency of pallor or discoloration in the liver of high-dose fetuses. Reduced ossification of cranial bones, sternebrae, pubes, metacarpals and metatarsals in mid- and high-dose groups were an indication of developmental retardation. In addition, fetuses from all three dose groups showed reduced ossification in the inter-parietal bone and an increase in angulated ribs over control values.

V. Core Classification: Core-Supplementary Data. A new study is required.

Maternal Toxicity NOEL = 150 mg/kg/day (LDT)
Maternal Toxicity LOEL = 550 mg/kg/day
Developmental Toxicity NOEL = not determined
Developmental Toxicity LOEL = 150 mg/kg/day (LDT)

Folpet toxicology review

Page _____ is not included in this copy.

Pages 12 through 18 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.