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

SEP - 3 1998

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Amine Oxide: Review of Toxicology Data

EPA Identification Numbers:

P.C. Code: 000439
DP Barcodes:D247460

MRID's: 44434907
Submissions: S545255

TO: Velma Noble/Jackie Campbell / PM Team 31
Regulatory Management Branch I
Antimicrobials Division (7510W)

FROM: Timothy F. McMahon, Ph.D. 8/25/98
Senior Toxicologist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510W)

THRU: Laura Morris 8/28
Team Leader, Team Two
RASSB
Antimicrobials Division (7510W)

and

Norm Cook, Chief 09/03/98
RASSB
Antimicrobials Division (7510W)

Action Requested: Review of a developmental toxicity study conducted with alkyl trimethyl ammonium bromide.

Background

The registrant (Proctor and Gamble Corporation), as part of an application to the Agency for registration of amine oxide as an antimicrobial active ingredient in dish detergent, submitted a developmental toxicity study conducted with alkyl trimethyl ammonium bromide. It is the registrant's opinionthat the study conducted with alkyl trimethyl ammonium bromide will substitute for a study which should be conducted with amine oxide. The executive summary of the reviewed study is shown below.

CITATION: Conzens, D. D., Edwards, J. E., Billington, R., and Clark, R. (1981) Effects of E9076 on Pregnancy of the Rat. Huntingdon Research Centre, Huntingdon, Cambridgeshire. Laboratory ID# 893-R/811, April 15, 1981. MRID 44434907. Unpublished

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44434907), alkyl trimethyl ammonium bromide, structurally related to amine oxide (E9076; purity not specified) was administered by gavage to 20 Crl:COBS CD (SD) BR female rats/dose at dose levels of 0, 6, 18, or 54 mg/kg/day from days 6 through 15 of gestation. On day gestation day 20, all surviving dams were sacrificed and necropsied.

Maternal clinical toxicity consisted only of increased post-dosing salivation (20/20 treated vs 0/20 controls) in the high-dose group. No treatment-related clinical signs were observed in the mid- and low-dose groups. No differences in bodyweight or food consumption were observed in any of the treated groups compared to the controls.

Pre-implantation loss was decreased (181%, $p<0.05$) and mean litter weights were increased (19%, $p<0.05$) in the high-dose groups. The numbers of corpora lutea, implantations, viable fetuses, and the extent of post-implantation losses were unaffected by treatment.

Based on the increase in post-dose salivation, **The maternal LOEL is = 54 mg/kg/day. The maternal NOEL = 18 mg/kg/day.**

No treatment-related effects on developmental parameters were observed.

A developmental LOEL was not observed. The developmental NOEL is >54 mg/kg/day.

This developmental toxicity study in the rat is classified as **unacceptable (§83-3(a))** and does not satisfy the guideline requirement for a developmental toxicity study in the rat. This study was intended to be used in place of conducting a developmental toxicity study with amine oxide. Although a maternal LOEL was observed at the 54 mg/kg/day dose level, the test material (Alkyl (C14) trimethyl ammonium bromide) is felt to be chemically dissimilar to amine oxide for purposes of toxicology testing. Therefore, the registrant is required to perform a developmental toxicity study using amine oxide as the test material.

DATA EVALUATION RECORD

E9076 (Structurally related to Amine Oxide)

Study Type: 83-3a; Effect of E9076 on Pregnancy of the Rat

Work Assignment No. 3-27 (MRID 44434907)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Guy R. Beretich, Ph.D.

Signature: Guy R. Beretich
Date: 7/29/98

Secondary Reviewer:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 8/14/98

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 8/14/98

Quality Assurance:
Steve Brecher, Ph.D.

Signature: Steven Brecher
Date: 8/6/98

Disclaimer

This Data Evaluation Record may have been altered by the Antimicrobial Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: T. McMahon, Ph.D. _____ Date: 8/24/98
Antimicrobials Division (7510W)

EPA Work Assignment Manager: T. McMahon, Ph.D. _____ Date: 8/24/98
Risk Assessment and Science Support Branch (7510W)
Antimicrobials Division

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - rat
OPPTS Number: 870.3700 OPP Guideline Number: §83-3a

DP BARCODE: D244775 SUBMISSION CODE: None
P.C. CODE: Not provided TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): E9076, (Purity not specified).

SYNONYMS: Alkyl (C14) trimethyl ammonium bromide.

CITATION: Conzens, D. D., Edwards, J. E., Billington, R., and Clark, R. (1981) Effects of E9076 on Pregnancy of the Rat. Huntingdon Research Centre, Huntingdon, Cambridgeshire. Laboratory ID# 893-R/811, April 15, 1981. MRID 44434907. Unpublished

SPONSOR: The Proctor & Gamble Company, Cincinnati, OH

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44434907), alkyl trimethyl ammonium bromide, structurally related to amine oxide (E9076; purity not specified) was administered by gavage to 20 Crl:COBS CD (SD) BR female rats/dose at dose levels of 0, 6, 18, or 54 mg/kg/day from days 6 through 15 of gestation. On day gestation day 20, all surviving dams were sacrificed and necropsied.

Maternal clinical toxicity consisted only of increased post-dosing salivation (20/20 treated vs 0/20 controls) in the high-dose group. No treatment-related clinical signs were observed in the mid- and low-dose groups. No differences in bodyweight or food consumption were observed in any of the treated groups compared to the controls.

Pre-implantation loss was decreased (181%, $p<0.05$) and mean litter weights were increased (19%, $p<0.05$) in the high-dose groups. The numbers of corpora lutea, implantations, viable fetuses, and the extent of post-implantation losses were unaffected by treatment.

Based on the increase in post-dose salivation, **The maternal LOEL is = 54 mg/kg/day. The maternal NOEL = 18 mg/kg/day.**

No treatment-related effects on developmental parameters were observed.

A developmental LOEL was not observed. The developmental NOEL is >54 mg/kg/day.

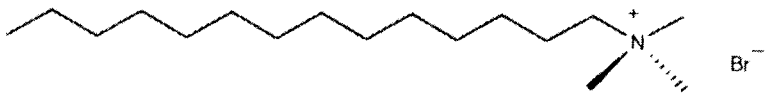
This developmental toxicity study in the rat is classified as **unacceptable (§83-3(a))** and does not satisfy the guideline requirement for a developmental toxicity study in the rat. This study was intended to be used inplace of conducting a developmental toxicity study with amine oxide. Although a maternal LOEL was observed at the 54 mg/kg/day dose level, the test material (Alkyl (C14) trimethyl ammonium bromide) is felt to be chemically dissimilar to amine oxide for purposes of toxicology testing. Therefore, the registrant is required to perform a developmental toxicity study using amine oxide as the test material.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Alkyl trimethyl ammonium bromide
   Description: Not specified
   Lot/Batch #: ECM BTS 272S1
   Purity: Not specified
   CAS #: Not provided
   Structure:



2. Vehicle: Distilled water

3. Test animals: Species: Rat
   Strain: Crl:COBS CD (SD) BR
   Age at mating: Not specified
   Weight at mating: 154 to 192 g
   Source: Charles River (U.K.) Limited, Margate, Kent
   Housing: 5 per cage in solid metal cages with wire-mesh top, front and floor.
   Diet: Spratts Laboratory Diet No. 1, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 56±8%
   Air changes: 13/hour
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): None specified

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: May 2, 1979 end: June 11, 1979

2. Mating: Rats were time-mated at the supplier's facility. The day of mating was determined by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug and was considered day 0 of pregnancy. Animals were delivered on day 1 of pregnancy.

3. Animal Assignment: Animals were assigned on a bodyweight basis to dose groups as indicated in Table 1.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
1 - Control	0	20
2 - Low	6	20
3 - Mid	18	20
4 - High	54	20

4. Dose selection rationale: A preliminary study was performed in which Alkyl trimethyl ammonium bromide was administered by gavage to 4 sexually mature, non-pregnant Crl: COBS CD (SD) BR rats/dose at dose levels of 0, 15, 30, 60, 120, or 240 mg/kg/day. Dosing lasted for 10 days, and all surviving rats were necropsied on day 15. At 240 mg/kg/day, all animals died or were killed for humane reasons three to six days after treatment commenced. Clinical signs were stated to be marked salivation, pale cold extremities, fur staining, diminished bodyweight gains (↓5% vs controls on day 4), anorexia and gastro-intestinal disturbances. At 120 mg/kg/day, clinical signs observed were stated to be marked salivation, fur-staining, diarrhea, reduced food consumption (↓41% vs controls for days 4 to 7), and diminished bodyweight gains (↓9% vs controls on day 7). At 60 mg/kg/day, observed clinical signs were stated to be salivation, reduced food consumption (↓14% vs controls for days 4 to 7), and slightly reduced bodyweight gain (↓4% vs controls on day 7). At 30 mg/kg/day, salivation was stated to have been observed in two animals and bodyweight gain was slightly retarded (↓1% vs controls at day 7). Effects at 15 mg/kg/day were limited to bodyweight loss (↓7% vs controls at day 7). No treatment-related effects were found at necropsy. Based on the results of these studies, 54 mg/kg/day was selected as the high dose for the subsequent oral developmental toxicity study in rats. Low- and mid-dose levels chosen were 6 and 18 mg/kg/day, respectively.

5. Dosage preparation and analysis: Alkyl trimethyl ammonium bromide was mixed with distilled water to 0.54% and serially diluted to achieve the lower concentrations. Solutions were prepared fresh daily. No concentration, homogeneity or stability analyses were performed.

6. Dosage administration: All animals received a dose volume of 1 ml/100 g bodyweight by gavage. All doses were administered once daily on gestation days 6 through 15. Dosing was based on the bodyweight determinations on gestation days 6, 10, and 14.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were observed daily for clinical

8

signs. Bodyweights were recorded on gestation days 1, 3, 6, 10, 14, 17, and 20. Bodyweight changes were not calculated. Food consumption was recorded for days 1-3, 3-6, 6-10, 10-14, 14-17, and 17-20. There were no abortions. On gestation day 20, all surviving animals were sacrificed and examined for macroscopic abnormalities. The reproductive tract was examined and the following data were recorded:

- pregnancy status
- number of corpora lutea
- number and distribution of live fetuses
- number of implantation sites
- numbers of embryonic deaths (early and late)
- litter weights

2. Fetal Evaluations - Each fetus was sexed, weighed and examined for external abnormalities. Half the pups were preserved in Bouin's solution for subsequent visceral examination. The remainder were fixed in methylated spirit, examined macroscopically and eviscerated. The fetuses were then cleared and alizarin-stained to allow for skeletal examination.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures

2. Indices: The following indices were calculated by the investigator:

Pre-implantation loss:
corpora lutea - # implantations/# corpora lutea x 100

Post-implantation loss:
implantations - # viable progeny/# implantations x 100

3. Historical control data: Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations - The high-dose group showed increased post-dosing salivation (20/20 treated vs 0/20 controls) throughout the treatment period. There were no deaths and no other treatment-related clinical signs.

2. Bodyweight - Bodyweight gains were decreased (↓14%) in the high-dose group during the first part of the treatment (Days 6-10; Table 2).

Table 2. Mean bodyweight changes (g) in animals dosed with Alkyl trimethyl ammonium bromide on days 6 through 15 of gestation.[a]

Interval	Dosage (mg/kg/day)			
	0	6	18	54
Pre-treatment Days 1-6	42.1	39.6	37.9	41.8
Treatment Days 6-10	22.3	22.4	20.1	19.2
Treatment Days 6-14	47.8	51	41.4	46.5
Post-treatment Days 14-20	73.8	64.4	72.5	76.4
Overall Days 1-20	163.7	155	151.8	164.7

a Data calculated by the reviewers from data in study report Table 4, page 28.

3. Food Consumption - Food consumption was unaffected by treatment.

4. Gross Pathology - There were no treatment-related gross pathologic findings noted in any of the dams.

5. Cesarean Section Data - Cesarean section observations are presented in Table 3. The pregnancy rate was not affected by treatment. Pre-implantation loss was decreased in the high-dose groups (↓81%, $p<0.05$). Mean litter weights were increased (↑9%, $p<0.05$). The numbers of corpora lutea, implantations, viable fetuses, the extent of resorptions/implantations and mean fetal weights were similar between control and treated groups.

Table 3. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	6	18	54
Animals Assigned (Mated)	20	20	20	20
Animals Pregnant	19	18	17	17
Pregnancy Rate (%)	(95)	(90)	(85)	(85)
Nonpregnant	1	2	3	3
Maternal Wastage				
Died	0	0	0	0
Died Pregnant	0	0	0	0
Died Nonpregnant	0	0	0	0
Aborted	0	0	0	0
Premature Delivery	0	0	0	0
Total Corpora Lutea(FTG)	229[b]	192	203	194
Corpora Lutea/Dam	12.1	10.7	11.9	11.4
Total Implantations(FTG)	202	175	185	190
Implantations/Dam	10.6	9.7	10.9	11.2
Total Litters	19	18	17	17
Total Live Fetuses	194	168	174	185
Live Fetuses/Dam	10.2	9.3	10.2	10.9
Total Dead Fetuses	NR	NR	NR	NR
Dead Fetuses/Dam				
Total Embryonic Deaths	8	7	11	5
Early	8	7	9	5
Late	0	0	2	0
Embryonic Deaths/Dam	0.4	0.4	0.6	0.3
Early	0.4	0.4	0.5	0.3
Late	0	0	0.1	0.0
Litters with Total Embryonic Deaths	0	0	0	0
Pre-implantation loss (%)	9.9	10.0	7.7	1.9*
Post-implantation loss (%)	4.0	3.5	5.2	2.6
Mean Litter Weight (g)	38.47	34.07	38.24	41.98*
Mean Fetal Weight (g)	3.77	3.69	3.75	3.86
Sex Ratio (% Male)[b]	47.0	45.2	51.0	51.4

a Data extracted from the study report Table 5 and Appendix 5, pages 29 and 39 through 42.
b Total Corpora Lutea, Total Implantations, Total Live Fetuses and Sex Ratio were calculated by the reviewers.
* Significantly different from controls at $p<0.05$.
FTG - Full term gestating females

(1

B. DEVELOPMENTAL TOXICITY Fetal evaluations included external, visceral, and skeletal examinations. A significant ($p<0.05$) increase in the number of fetuses with normal sternebrae (73% of treated vs 52% of controls) and a concomitant decrease in the number with abnormal sternebrae (27% of treated vs 48% of controls) occurred in the mid-dose group. However, this effect was not dose-dependent and therefore, not considered treatment related. There were no other significant differences in external, visceral, or skeletal malformations or variations.

External, visceral, and skeletal fetal findings are presented in Tables 4a, 4b, and 4c below.

Table 4a. External examinations[a]

Observations [b]	Dose (mg/kg/day)			
	0	6	18	54
#Fetuses(litters) Examined	194(19)	168(18)	174(17)	185(17)
#Fetuses(#litters) with Malformations	0(0)	0(0)	0(0)	1(1)
#Fetuses(#litters) with Variations	NR	NR	NR	NR
Vertebral agenesis	0(0)	0(0)	0(0)	1(6)

a Data extracted from the study report Table 6 and Appendix 6, pages 30 and 43 through 50.
b Values for individual observations are as follows: %fetal(%litter). %litter calculated by the reviewers and litter rounded to nearest whole number.

Table 4b. Visceral examinations[a]

Observations [b]	Dose (mg/kg/day)			
	0	6	18	54
#Fetuses(litters) examined	97(19)	83(17)	87(17)	91(17)
#Fetuses(#litters) with Malformations	0(0)	0(0)	0(0)	0(0)
#Fetuses(#litters) with Variations	2(2)	2(2)	1(1)	5(5)
Intercostal Vein Irregularity	1(5)	0(0)	1(6)	0(0)
Renal Pelvic Cavitation	1(5)	0(0)	0(0)	1(6)
Carotid and Subclavian Arteries Arising from Aortic Arch	0(0)	1(6)	0(0)	1(6)
Ocular Hemorrhage	0(0)	1(6)	0(0)	0(0)
Displaced Testis	0(0)	0(0)	0(0)	1(6)
Atelectasis	0(0)	0(0)	0(0)	1(6)
Intra-abdominal Hemorrhage	0(0)	0(0)	0(0)	1(6)

a Data extracted from the study report Table 6 and Appendix 6, pages 30 and 43 through 50.
b Values for individual observations calculated by the reviewers are as follows: %fetal (%litter).

Table 4c. Skeletal variations[a]

Observations	Dose (mg/kg/day)			
	0	6	18	54
#Fetuses (#litters) examined	97(19)	85(18)	87(17)	93(17)
#Fetuses (#litters) with Malformations	0(0)	0(0)	0(0)	0(0)
#Fetuses (#litters) with Variations	NR	NR	NR	NR
13 Ribs[b]	90(NR)	97(NR)	94(NR)	92(NR)
14 Ribs[b]	10(NR)	3(NR)	6(NR)	8(NR)
Normal Sternebrae[b]	52(NR)	67(NR)	73*(NR)	68(NR)
Variant Sternebrae[b]	48(NR)	33(NR)	27*(NR)	32(NR)
Variant Centrae[c]	2(5)	5(22)	2(12)	3(18)
Reduced Vertebral Arches[c]	2(11)	2(11)	3(12)	0(0)

a Data extracted from the study report Tables 6 and Appendix 6, pages 30,31 and 43 through 50.

b Values for individual observations rounded to nearest whole number by the reviewers are as follows: %fetal, (%litter).

c Values calculated by reviewers

NR = not reported.

* Significantly different from controls at $p<0.05$.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS The study author concluded that intragastric administration of Alkyl trimethyl ammonium bromide at 54 mg/kg/day to pregnant rats during days 6-15 of gestation provoked increased salivation and slight, initial retardation of weight gain. Weight gain was also slightly retarded at 18 mg/kg/day. Pregnancy rate was not affected. Pre-implantation loss was significantly lower ($p<0.05$) at 54 mg/kg/day. Lower doses exhibited no maternal toxicity. Alkyl trimethyl ammonium bromide had no conclusive effect on litter parameters or embryonic or fetal development.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following intragastric administration of Alkyl trimethyl ammonium bromide (purity not specified) at 0, 6, 18, and 54 mg/kg/day to pregnant rats during days 6-15 of gestation, an increase in post-dosing salivation was observed in the high-dose group (20/20 treated vs. 0/20 controls). Food consumption and bodyweight gains were not affected.

   Pre-implantation loss was decreased (181%, $p<0.05$) and mean litter weights were increased (19%, $p<0.05$) in the high-dose groups. The numbers of corpora lutea, implantations, viable fetuses, and the extent of post-implantation losses were unaffected by treatment.

   Maternal LOEL = 54 mg/kg/day (increased post-dose salivation).
   Maternal NOEL = 18 mg/kg/day.

2. DEVELOPMENTAL TOXICITY: No treatment-related developmental toxicity was observed.

   a. Deaths/Resorptions: No effects on fetal viability were observed.
   b. Altered Growth: No effects on fetal growth were observed.
   c. Developmental Variations: No treatment-related variations were observed.
   d. Malformations: No significant difference in the occurrence of malformations was observed.

   Developmental Toxicity LOEL = Not observed.
   Developmental Toxicity NOEL > 54 mg/kg/day.

   The developmental toxicity study in the rat is classified as **Unacceptable (§83-3(a))** and does not satisfy the guideline requirement for a developmental toxicity study in the rat.

C. STUDY DEFICIENCIES Aside from certain deficiencies noted in this study (such as purity of the test compound, formulation concentration, homogeneity, and stability data, and

historical control data), the main deficiency relates to the use of alkyl trimethyl ammonium bromide as a substitute for amine oxide. The use of alkyl trimethyl ammonium bromide in place of amine oxide is unacceptable based on chemical dissimilarities betwen the two compounds. The registrant must fulfill the developmental toxicity data requirement for amine oxide by conducting a study with amine oxide as the test material.

Mean food consumption (g/rat/day) was occasionally significantly decreased in high-dose males (↓5-10% vs controls; 5/8 periods; $p<0.01$ or 0.001) and high-dose females (↓4-7% vs controls; 6/8 periods; $p<0.05$-0.001). There were no significant differences in food utilization between the treated and control groups.

There was an increase in bilateral cataracts/opacities in the high-dose male rats (↑167% vs controls) at final necropsy. During study weeks 66 to 104, the incidence of bilateral cataract/opacities in the high-dose females was 2.4-6.4% vs 0% in the controls. The rats were examined by two different ophthalmologists, both of whom did not attribute any findings to the treatment. However, when compared to the sub-chronic toxicity study (MRID 44475203), in which 12/20 males and 8/20 females had lenticular cataracts/opacities in the 0.4% treatment groups at 13 weeks, it may be possible that a treatment-related effect is being observed.

No treatment-related microscopic postmortem differences were observed between rats in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

In conclusion, the dose levels employed in this study were adequate to characterize the chronic toxicological potential of amine oxide in both sexes of rats. Chronic toxicity was characterized in the high-dose males and females by reduced body weight and food consumption, and increased bilateral opacities/cataracts.

The chronic LOEL is 0.2% [87.4/107 (M/F) mg/kg/day] based on decreased body weight and ophthalmological opacities/cataracts. The chronic NOEL is 0.1% [42.3/52.6 (M/F) mg/kg/day].

Under the conditions of this study, amine oxide did not show any signs of oncogenicity it CD rats.

The submitted study is classified as **acceptable (§83-5(a))** and satisfies the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

C. Study deficiencies -The ophthalmoscopic examination data were not submitted in summary form.