UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: 6/15/78

SUBJECT: 10182-EUP-11. Baquacil Swimming Pool Sanitizer [containing poly (hexamethylene biguanide hydrochloride)]. Experimental Use Permit Application for the evaluation of Baquacil in recreational swimming pools. Caswell # 676

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FROM: William Dykstra, Ph.D
Toxicology Branch WHD 6/15/78

TO: Libby Zink
Special Registration Section
Room 315 (d)

Registrant: ICI Americas Inc.
Safety and Environmental Affairs
Wilmington, Delaware 19897

Recommendations:

1. The [illegible]ot toxicologically supported because of the incidence of hep[illegible]umors noted in Section C-16 and Section C-17 (points #4 + 5). C-18

2. Mutagenic assessment (multi-test) is required for registration.

3. Analysis of the active ingredient for nitrosamine content is required for registration.

4. The statistically significant incidence of hepatic tumors in the high-dose mice in the PHMB 80 week Skin Painting study in mice (Report No. CTL/P/331), Section C-16 may trigger an oncogenic RPAR criterion.

5. The statistically significant incidence of hepatic tumors, which appears to be dose-related, in the PHMB: Life-Time feeding study in mice (Report CTL/P/332), Section C-17, may trigger an oncogenic RPAR criterion. C-18

6. Labeling is adequate for TOXICITY CATEGORY I: DANGER, based on eye and skin effects. This p[illegible] should be considered as a candidate for restricted u[illegible]

7. Review of the Toxicology Data has established that the classification of many studies is supplementary or inadequate. These categories of classification do not support the registration and the studies are required to upgraded by addressing the unresolved questions relating to them or must be repeated for registration. A summary of the classification of the studies is as follows:

1. Section C-1. Toxicological Report

   a. Acute oral LD_{50} Rats - Supplementary Data

   b. Acute oral LD_{50} Rats - Supplementary Data

   c. Acute oral toxicity - Supplementary Data

   d. Repeated oral toxicity - Supplementary Data

   e. Intraperitoneal Toxicity - Supplementary Data

   f. Skin application - Inadequate Data — Supplementary ACS 5/14/84

   g. Eye irritation - Inadequate Data — Supplementary ACS

2. Section C-2. Toxicological Report

   a. Skin application - Supplementary Data

   b. Eye irritation - Supplementary Data

3. Section C-3. Toxicological Report

   a. Repeated application - Supplementary Data

4. Section C-4. Acute Dermal Toxicity and Skin Irritation Effects - Supplementary Data

5. Section C-5. Toxicological Report. Skin Sensitization - Inadequate Study

6. Section C-6. Photoirritation Test - Inadequate Study

7. Section C-7. Photoreaction patch test - core minimum data

8. Section C-8. Subacute Dermal Toxicity in the Rabbit - Supplementary Data

9. Section C-9. PHMB, Teratogenicity Study in the Mouse - Core Minimum Data, but NEL not established for delayed ossification.

10. Section C-10. Teratologic Evaluation of IL-780 in Rabbits - Inadequate Study

11. Section C-11. Baquacil SB. A teratology Study in rat - Core Minimum Data. Teratologic NEL is considered to be 1000 ppm PHMB in the diet during gestation of rat.

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12. Section 12 - Core Minimum Data as part of Section C-11.

13. Section 13. A Three generation Reproduction Study in rats with 20% PAMB - Core Minimum Data . Reproductive NEL is 1300 ppm for reproductive parameter.

14. Section C-14. 90 day oral toxicity of Antibacterial 9073 - Albino Rats-Supplementary Data.

15. Section C-15. 90 day oral toxicity of Antibacterial 9073 Beagle Dogs - Core Minimum Data. NEL is 5500 ppm in diet.

16. Section C-16. PHMB. 80 Week Skin Painting Study in Mice. Core Minimum Data but PHMB produces liver tumors in high-dose group above levels of controls. This may trigger an oncogenic RPAR criterion.

17. Section C-17. Test Experiments concerning the effects of prolonged oral intake of product Vantoul IB - Inadequate Data.

18. Section C-18. PHMB. Life-time feeding Study in the mouse - Core Minimum Data but PHMB produces liver tumors (dose-related response) above controls. This may trigger an oncogenic RPAR criterion.

19. Section C-19. PHMB. A long term feeding study in rats. Core minimum Data. NEL is considered to be 200 ppm of PHMB in diet of rats.

20. Section C-20. 26 week Toxicity Study in Dogs with 20% PHMB. Core Minimum Data. A NEL was not established in this study.

21. Section C-21. Vantocil IB: Absorption and excretion studies in the rat - Supplementary Data.

22. Section 22. Characterization of the Urinary polymer - related material from rats - Core Minimum Data.

Product: Baquacil

Ingredient	Product Weight
poly(hexamethylenebiguanide hydrochloride)	20.0
Inerts	80.0

Directions for Uses:

To dose and maintain: To control microorganisms in pool waters, adjust pool pH to 7.0 - 8.0 and add Baquacil at a 50 ppm level (10 ppm active) The concentration of Baquacil should be checked weekly with The Baquacil Test Kit and additional Baquacil added as indicated to bring the concentration back up to the 50 ppm level. The frequency of additional doses of Baquacil will depend on pool load and amount of organic debris.

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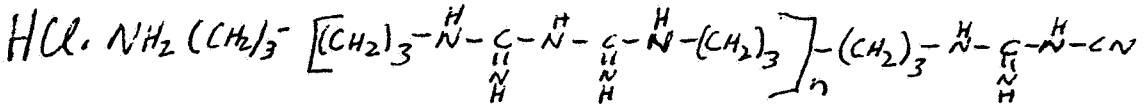
Areas Treated

Baquacil will be tested in Florida, Pennsylvania and Tennessee. Total pool capacity to be tested in each state is 174,394 gallons for Florida (4 locations), 345,000 gallons for Pennsylvania (3 locations) and 237,000 gallons for Tennessee (3 locations).

STRUCTURE:



$n = 4.5-6.5$

M.W. = (219.7)n

Review

Section C. Toxicity Data Volume 3 of 8.

Section C-1

Toxicological Report. Antibacterial 9073, TR/558, October 26, 1966 test material 25% Aqueous solution of PHMB Oral administration.

Rats

a. Single doses of 4.0 gm/kg body weight of test material (equivalent to 1.0 gm/kg of PHMB) were given by stomach tube to one group of 3M + 3F young adult rats (strain and BW not specified). Observation was for 7 days.

Results: One female rat died

$LD_{50} > 1.0$ gm/kg BW of RHMB

Toxic signs: piloerlltion, failure to groom, mild scouring

Necropsy: Generalized congestion with gastric distention and hemorrhage, thymic lympholysis.

Body Weight: Not reported

Classification: Supplementary Data Tox Cat: III CAUTION

1. Raw data not submitted

2. Strain, bodyweight of rats unspecified

3. Observation for only 7 days .

b. A single dose of 2.0 gm/kg of test material was given to 3 female rats (strain, age BW not specified). Observation for 7 days

Toxic signs: None observed, No deaths

Necropsy: Organs appeared normal

Bodyweight: Not reported

Classification: Supplementary Data Tox Cat. III CAUTION

1. Observation for 7 days instead of 14 days.

2. Raw data not submitted

3. Sex, age and BW of rats unspecified.

c. One group of 3M + 3F rats (strain, age & BW unspecified) were given 40 gm/kg of test material and survivors killed the following day. One male rat died overnight.

Necropsy: Severe generalized congestion with dilatation of the stomach and mucosal hemorrhage. Microscopy revealed gastric inflammation, ulceration and thymic lympholysis but no other specific lesions.

Classification: Supplementary Data

1. Raw data not submitted

d. Twenty one consecutive daily doses were given to one group of 7M + 7F rats where survival allowed. Initially 1.0 gm/kg was given but subsequently 0.5 gm/kg were administered. Survivors were observed for 7 days after the last dose. Blood was examined at the outset and 24 hours after the final dose.

Results: Deaths occured throughout the period of treatment and only 4 males and 2 females survived 21 doses. One of these male rats died during the observation period. Toxic signs not reported.

Necropsy: Gastrointestional irritation severe gastric hemorrhage ulceration, peritonitis, thymic atrophy, generalized congestion. Microscopic examination of the major organs revealed changes of a non-specific nature consistent with gastrointestinal inflammation.

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Classification: Supplementary DATA

1. Raw data not submitted.

e. Intraperitoneal Injection

Single doses ranging from 25-500 mg/kg BW were given to groups of three male and three female rats and the animals observed over a 7 day period if survival allowed. From the resulting mortality the acute i.p. LD_{50} is estimated to be between 50-100 mg/kg for both sexes (approximately 12.5-25 mg/kg of PHMB).

Results

Male rats given 100 mg/kg or more died in coma within an hour of dosing but only one male given 50 mg/kg died, and that after three days. The mortality pattern was not so clear-out in females although those given 250 mg or more also died within an hour of dosing. Deaths among animals given 100 mg/kg occurred over 5 days and a single death among animals given 50 mg/kg occurred 24 hours after dosing. No deaths occurred at the lowest dose level in either sex. Toxic effects appeared to be due to serve abdominal irritation and no specific signs were noted. Gross findings post-mortem were consistent with peritonitis and microscopic examination of the major organs showed only non-specific lesions consistent with peritonitis.

Classification: Supplementary DATA

1. Raw data, dose levels etc. not submitted.

Skin application

The supplied liquid was applied beneath an occlusive dressing to the shorn backs of three female rats for three alternate 24-hour periods. After each 24-hour application the dressing was removed and the skin washed with soap and water, greatly dried and exposed to the air for 24 hours until the next application.

Results:

A single application caused focal ulceration which worsened after the second and third applications by which time there was pronounced overall edema. There were no specific systemic effects.

Classification: Inadequate Study Tox. Cat. I DANGER

1. Amount of material applied not specified.

2. Abraded skin was not tested.

3. Sufficient animals animals were not used.

4. Scoring system and raw data not submitted.

g. Eye Irritation

A single drop on the supplied liquid caused severe inflammation and corneal damage in three rabbits. The condition was partly resolved in two animals after one week but the third animal appeared to be permanently blinded in the treated eye. This instillation was repeated but the eyes were irrigated with saline five seconds later. Only slight inflammation followed and the eyes were virtually normal by the third day.

Classification: Inadequate Study Tox Cat. I DANGER

1. Amount of material applied not specified.

2. Sufficient animals were not used.

3. Scoring system and raw data not submitted.

Section C-2

Toxicological Report. The irritant properties of Vantocil I.B. (Imperial Chemical Industries Limited. Industrial Hygiene Research Laboratories. Report HO/IH/T/704A. Division: Dyestuffs July 24, 1969)

Experimental

Vantocil I.B. contains 20% of PHMB. The working solution contains 0.04% of the active ingredient.

Skin Application

a. Occluded

Vantocil I.B. was diluted with water to the working strength solution (0.04% of the active ingredient) and 0.1 ml of this solution was applied to the shorn backs of each of 5 female rats (Alderley Park strain) on alternate days. Immediately after each application the treated area was covered with polythene, which was kept in place by adhesive plaster. The animals were kept in separate cages. Twenty-four hours after each application the dressings were removed, the treated area cleansed with methylated spirits and the condition of the skin recorded.

Six applications of the substance were made and no irritation was observed at anytime nor was there any evidence of systemic toxicity.

b. Non-occluded

In another experiment Vantocil was similarly applied to the shorn backs of another 5 female rats, but the treated areas was left uncovered. Plastic collars were placed around the necks of the animals to prevent them cleaning the treated area. Six applications were made and no irritation was observed, and again there was no evidence of systemic toxicity.

Classification: Supplementaty Data

1. Raw Data results, abraded skin, scoring system not submitted.

Eye Irritation:

25% solution of PHNB is known to be highly irritant to eyes (TR/558). 0.1 ml of Vantocil was instilled into one eye of three rabbits, and after 20 seconds with an intermittent stream of physiological saline. Only slight inflammation resulted and no corneal ulceration was detected. These changes resolved in three days.

In another experiment 0.1 ml of the working strength solution was instilled into one eye of each of three rabbits which were then observed for several days. There were no immediate or delayed irritant effects.

Classification Supplementary Data

1. Scoring system not submitted

2. Tables, raw data, etc., not submitted.

Section C-3

Toxicological Report. Further Studies on the irritant effects of Vantocil I.B. (Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories, Report No,: HO/IH/T/704B; Division Dyestuffs, November 21, 1969)

Summary of Experimental Results

Repeated application of Vantoxil IB to rat skin was not irritant unless the concentration exceeded 5% of PHMB. At this concentration there was no effect on the eyes of rabbits.

Classification: Supplementary Data

1. Only summary was submitted, not actual reports.

Section C-4

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Report No. CTL/T1057. Baquacil SB: Acute Dermal Toxicity and Skin Irritation Effects (Imperial Chemical Industries Limited, Central Toxicology Laboratory, Division: Organics; Div. Ref: Baquacil SB, ADGM 3152 Org/100/77, CTL Ref: 70223 October 6, 1977.

Healthy rats of the required weight were selected (Alderley Park, SPF, albino strain, initial BW 150-200 gm) from stock and caged individually. The hair was removed from the back and flanks with. electric clippers 18 hours before application of the test material Prior to use each animal was inspected and those with any obvious skin lesion rejected. Doses of 2.5 ml/kg and 5.0 ml/kg were applied to 10 male and 10 female animals. The material was topically applied to each animal from a syringe, and spread over an area approximately one inch square. The treated animals were caged alone for 24 hours, after which the dressing was removed and the skin and surrounding hair sponged thoroughly with warm detergent solution (1% aqueous Cetrixmide - ICI limited). After rinsing the animals were dried with absorbent cotton wool. The test animals were then caged in groups of 5, and observed for a period of 7 days. Any signs of toxicity and irritation were noted daily throughout the observation period.

Results: No deaths, although all showed severe skin irritation effects.

LD_{50} >5.0 ml/kg

Necropsy: Not performed.
Bodyweight: A few animals lost weight during observation period.

Classification: Supplementary Data

1. Abraded skin was not tested.

Section C-5. Toxicological Report

Skin Sensitization Tests on PHMB solutions, Vantocil IB (20% in water) and Antibacterial 9073 (25% in water)(Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories Report No. TR/684, Division: Dyestuffs, January 6, 1969.

Method

Each ear of mix Porton strain albino guinea pigs was treated once a day for three consecutive days with 0.1 ml of Antibacterial 9073.

One week after the first application of material to the ears, both flanks of the six guinea pigs and of four control Portion strain albino-guinea pigs were closely clipped. 0.2 ml each of Antibacterial 9073, 50% Antibacterial 9073 in dimethylformanide, and 10% Antibacterial 9073 in dimethylformamide were applied, as separate 1 cm diameter circular areas on the fur clipped flanks of the test animals and of the controls. One day later the test areas were examined for signs of erythema.

Results:

On the patches on the ear treated animals which had received 100% Antibacterial 9073 in diemthylformanide slight to moderate erythema was noted. This same effect was noted on the controls and is therefore, an irritant effect on the skin.

Classification: Inadequate Study

1. At least 10 applications of test is required before challenge to assess allergic response.

2. Positive controls were not tested.

Section C-6 Photoirritation

Test: PH173/8. Biocide DS4961 - Baquacil SB (Environmental Safety Division, Unilever Research Colworth, Welwyn Laboratory project No. 99699, December 2, 1973.

10 Weanling male rats were treated with 0.1 ml was applied to clipped dorsal skin once daily for 4 days and the area was radiated with UVC (Blacklamp) for three hours daily. The treatment groups were as follows:

A. 0.005% 8-MOP in 10% ethanol + UV for 3 hours.

B. 0.005% 8-MOP in 10% ethanol + NO UV.

C. 25% Biocide DS 4961 in Dist. H_2O + UV for 3 hours.

D. 25% Biocide DS 4961 in Dist + NO UV.

E. 10% Biocide DS 4961 in Dist + UV for 3 hours

F. 10% Biocide DS 4961 in Dist + NO UV.

Results

Biocide DS 4961 does not exhibit significant photoirritancy. Brocide DS 4961 appears to have a very strong irritant potential.

Classification: Inadequate Data

1. Positive controls were not tested.

2. Only male rats were used.

3. Only 4 instead of 10 applications.

Section C-7 Photo-reaction Patch Test using Natural Sunlight (Hill-Top Research, project No. 76-165-72; June 10, 1976).

Test Materials:

Sample Code	Lab Code	Preparation for Test
Paint Control	A	Swatches painted & dried
Bronopol, Batch No. CT101237C	B	1:100 in water
Bacquacil SB, ADGM 3429	C	1:20 in water
DS 5199 (paint)	D	Swatches painted & dried
Vancide TH	E	1:95 in water
Sodium lauryl sulfate	F	Diluted to 0.01% in water

Procedure

The procedure used was an adaptation of the repeated patch test procedure of Draize. Thirty male and Thirty female panelists were enrolled for this study, of whom 25 completed the program. The four panelists who dropped from the test did so for personal reasons. The test patch consisted of a 20 X 20 mm square of Webril affixed to a 40 X 40 mm adhesive square. The swatches were moistened with 0.4 ml of test solutions B,C,E and F just before application to the panelists. To increase the skin penetrating properties of these sampler, SLS was added to these solutions to provide a final concentration of 0.01% SLS in the patch solution.

For application of Sampler A and D, paint was applied to the Werbil swatches. These were allowed to dry and were applied to the skin by means of the adhesive square used for the liquid samples. Just before application of samples A and D the skin sites were prepared by placing one or two drops of 0.01% SLS on the test site. Each of the test solutions was evaluated on each panelist. The patches, one of each of the test materials, were applied to each upper arm of each panelist (three patches to one arm and three patches to the opposite arm).

Application of all samples were made on Monday, Wednesday and Friday for four successive weeks to panelists 1-12 and for three successive weeks to panelists 13-30. The patches were applied at noon each day and were removed by the panelists 24 hours after application. Immediately after patch removal the test sites were exposed to the direct rays of mid-day sun for one hour. Applications were made to the same (0) sites unless strong reactions were produced, in such cases applications were made to two fresh sites which were designated M and M-1 sites. The patches applied to both of these sites were identical to those applied to the (0) sites.

The M-1 sites remained covered with the light-occlusive patch until the sites were scored just before the next patch application. If strong reactions were obtained with either of these patches, the patch was omitted for the remainder of the study. Some panelists who reacted strongly to paint sample D were patched on a new site with a piece of filter paper coated with the paint and thoroughly dried before application. These patches were designated M-2 Challenge applications of test materials A,B,C,E and F were applied to fresh sites (A) and previously patched sites (0) on Monday of Week 6 of the test. Sample D was not applied at challenge. Reactions to all patches were scored prior to the 2nd through 9th applications and on Monday following the 9th application. Reaction to the challenge applications were scored 48 and 96 hours following application.

Results

Type of reaction	Sample B	Sample D
Primary irritation or skin fatigue	13 panelists	5 panelists
Probably sensitized panelists No.	6, 17, 14, 22	3,4,8,10,7,15, 16,24,30
No irritation	8 panelists	10 panelists

Samples A,C,E and F (Baquacil SB & Vancide TH) were essentially non-irritating and did not induce sensitization when evaluated on 26 panelists. Samples B and D induced contact senisitization in about 15% and about 42%, respectively, of the 26 panelists evaluated. There was no indication at any time that any of the test materials were phototoxic or photoallergenic.

Classification: Core Minimum Data

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Section C-8. Vantocil IB Subacute Dermal Toxicity Study in the Rabbit (Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories, ICI Centre: Toxicology Bureau, Report No: HO/IH/P/22 May, 1972).

Test Material: Vantocil IB

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A group of six female albino rabbits (BW, 1.5 - 2.5 kg) had 1.0 of a 12,000 ppm solution of Vantocil IB applied daily to their shorn backs for 23 hours. The skin was then washed with soap and water and the solution was re-applied one hour later. A total of 21 daily applications was made. The skin was not occluded, oral contamination being prevented by means of a plastic collar. Three control rabbits were used. At the end of the experiment the rabbits were killed. Blood was taken hematological examination and liver, adrenal, kidney, spleen, ovary, uterus, heart, thymus and lung were examined histopathologically.

Results:

No signs of systemic toxicity or skin irritation were noted during the experimental period and the test animals gained weight at the same rate as the controls. There were no signs of organ damage when the animals were examined at autopsy and histopathological examination of tissues did not reveal any changes attributable to treatment. Hematological findings indicated no significant differences between test and control animals.

Conclusion: No organ weights, histopathlogical reports were submitted.
Classification: Supplementary DATA (a) Actual data not submitted

Section C-9 PHMB, Teratogenicity Study in the Mouse (Central Toxicology Laboratory, Report No: CTL/P/335, April, 1977).

Test Material: 20% Aqueous solution of PHMB.

Groups of at least 21 pregnant Alderley strain pregnant mice were dosed orally by gavage with 0(control), 10,20 or 40 mg/kg on days 6 to 15 inclusive of pregnancy. On day 18, the animals were killed by cervical dislocation. The abdomen of each mouse was opened and the intact uterus examined for numbers of live fetuses and resorptions. The uterus was dissected and the fetuses removed. Resorptions were then classified as early or late, being identified as the latter when fetal tissues were distinguishable. The following material tissues were submitted in formal corrosive form 21 pregnant control and 21 pregnant high dose animals for histological examination: lung, liver, kidney, ovary, uterus, placenta, stomach, duodenum, jejunum, ileum and mesenteric lymph node.

Maternal tissues from the remaining animals in all groups were examined macroscopically. The above listed tissues were microscopically examined. Data on litter and fetal parameters, i.e. number of resorptions, fetal weight and litter weight, were collected.

Alternate fetuses from each litter (starting randomly) were eviscerated and fixed in 70% methanol, the viscera being macroscopically examined for abnormalities. These fetuses were stained with Alizaum Red for subsequent skeletal examination. During this examination ossified bones were examined both for abnormalities and hindlimb digits was assessed on an 8 point scale (1 being the best ossified) although all individual bones were examined. The remaining fetuses were preserved and decalcified in Bouin's fixative and examined by the "Wilson" technique. Twenty litters per group were examined in detail and the remaining fetuses preserved in case of untoward results.

Data from test groups were compared to control groups by the appropriate statistical comparisons.

Results

Mean maternal body weight was similar in the control, 10 mg/kg and 20 mg/kg groups. There were more variable individual gains in the 40 mg/kg group than in the other groups, resulting in a slightly reduced mean body weight gain which was not statistically significant.

Food consumption was similar for all groups. Pathology was unremarkable (although pathology and histopathology data was not submitted) according to the report. Litter and fetal parameters were similar for all groups. Soft tissue anomalies were unremarkable. Skeletal examination revealed the following related anomalies:

Description	Group 1		Group 2		Group 3		Group 4	
					Double the controls			
Skull	No.	%	No.	%	No.	%	No.	%
wide fontanelle	9	8.4	10	9.3	29	25.7	24	20.9
frontals partially ossified	1	0.9	4	3.7	12	10.6	14	12.2
Sternbrae								
					Double the controls			
5th partially ossified	28	26.2	45	41.7	56	49.6	38	33.0
3rd misaligned	7	6.5	18	16.7	16	14.2	21	13.9
4th misaligned	12	11.2	25	23.1	29	25.7	21	18.3
5th misaligned	8	7.5	21	19.4	18	15.9	14	12.2
Hindlimb								
					Double the controls			
Assessment of Ossification Grade 4	6	5.6	17	15.7	11	9.7	16	13.9

The incidence of litters with wide fontanelles was as follows:

Group	1	2	3	4
Wide fontanelles	6	5	10	7

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Conclusion:

On the basis of these results, marginal retardation of ossification appears to occur at each treatment level (10, 20 and 40 mg/kg). However the test material is not teratogenic. The NEL for delayed ossification has not been established in this study.

Classification: Core minimum

(a) Necropsy and histopathology data were not submitted for dams, as reported in results.

Section C-10

Teratologic evaluation of IL-780 in Rabbits (Food and Drug Research Laboratories, Inc.; Report No. 5022; August 6, 1976).

Test material: IL-780; A.D.G.M. 5642; TC NO. 7631; clear pale yellow liquid.

Pregnant adult, New Zealand White female rabbits received from Day 6 and continuing daily through Day 18, doses of 0, 10, 40 and 160 mg/kg of test material by oral intubation in 1.0 ml/kg of water. Body weights were recorded on Days 0,6,12,18 and 29 of gestation. All animals were observed daily for appearance and behavior. On Day 29 of gestation, dams were ethanized and uterine contents examined. Soft tissue and skeletal examinations were conducted on the fetuses.

Results

In the three litters examined at the high dose level (160 mg/kg) the following adverse findings were reported: percent complete resorptions (50% vs 15.4 for controls), live fetuses/dam (3.33 vs 7.8 for controls), average body weight gain of dams. The administration of 10 and 40 mg/kg of test material had no clearly discernible effect on nidation or on maternal or fetal survival. The numbers of abnormalities seen in either soft or skeletal tissues of the treated groups did not differ from the number occurring spontaneously in the sham-treated controls. However, only 3 litters were examined at 160 mg/kg dose level.

Conclusion :

No terata were observed as a result of treatment. The administration of 160 mg/kg resulted in maternal toxicity which resulted in impaired implantation, increased early fetotoxicity and increased complete resorptions. However, only 3 litters were examined at this dose level.

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No abnormalities were seen in either the soft or skeletal tissues of the treated groups but the study is regarded as inadequate.

Classification: Inadequate Data. Minimum Data ACS 5/15/84

1. Only 3 litters observed at 160 mg/kg dose level.

2. Toxicology Branch requests that the FDRL lab submit historical data for the controls especially anomalies of soft and skeletal tissues.

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3. It is necessary for registrant to identify test material.

Section C-11

Baquacil SB.

A teratology study in the rat by dietary administration (Report No. C TL/P/1262, July 1976).

Test Material: Baquacil SB: 20% Aqueous solution of PHMB.

Groups of at least 20 pregnant Aderley Park female rats were fed diets containing 0 (vehicle control), 200, 1000 or 2000 ppm of PHMB, the active ingredient of Bacquacil SB, throughout gestation. A group fed 2900 ppm aspirin acted as a positive control. On day 20, animals were killed by cervical dislocation until at least 20 pregnancies in each group were established. Fetuses were removed by cesarian section and the uterus examined for resorptions. Resorptions were classified as early or late, being identified as the latter when fetal tissues were distinguishable. If any fetus was abnormal, its placenta together with maternal heart, lung, liver, kidney, adrenal, ovary and uterus were preserved in formol corrosive for histological examination. These maternal tissues were also routinely examined macroscopically after cesarian section. Fetal position , viability, sex and weight were recorded. Alternate fetuses from each litter were eviscerated and fixed in 70% Alcohol, the viscera being microscopically examined for abnormalities. These fetuses were stained with Alizarin Red for subsequent examination for skeletal effects. The remaining fetuses were fixed and decalcified in Bowin's fluid for at least 10 days prior to examination. Sections were made through the head and thorax according to the technique of Wilson. The abdomen was examined by dissection but sections were made through the kidneys to examine their internal structure.

Statistical comparison between control and treatment groups were made by appropriate methods.

Results:

There were no adverse clinical effects in any group. Mean maternal bodyweight was reduced ($P<.01$) significantly in the groups fed 1000 or 2000 ppm PHMB. The effect was more marked in groups fed the aspirin diet where the mean weight gain over the first week was only one gram because a number of animals lost weight while weight gain in the remainder was minimal. Food consumption was significantly reduced in animals receiving 1000 ppm or 2000 ppm PHMB or 2900 aspirin. Maternal microscopic findings revealed an enlarged and hemorrhagic thymus in one female which had received 2000 ppm PHMB. No other maternal abnormalities were seen.

The mean number of inplantations per litter was significantly reduced only for the group fed 2900 ppm aspirin suggesting that some pre-implantation loss of embryos had occurred. The number of early resorptions was significantly increased ($P<.01$) in the aspirin group indicating embryotoxicity. There was also a significant increase in the number of early resorptions in the 1000 ppm PHMB group, but this was not a dose related effect. There was no increase in late resorptions in any group. The mean number of fetuses per group was significantly reduced for the aspirin-treated animals only. The fetal weight and litter weight was reduced significantly ($P<.01$) for aspirin group but not for PHMB groups. Sex ratio was similar in all groups. Five abnormal fetuses were observed on gross examination. Gastroschisis was found in one fetus from the 200 ppm PHMB group, while umbilical hernia was seen in one fetus from the 2000 ppm PHMB group and in one from the aspirin-treated group. Exencephaly and spina bifida were seen in two fetuses from the same litter in the aspirin-treated group and was associated in one with slight hydrocephalus and a shortened neck which was displaced into the head, thus causing distortion of the brain. There was an increase in subcutaneous hemmorrhages in the aspirin-treated fetuses, although this was possibly due to reduction in size which could have rendered the fetus more susceptible to mechanical damage. Eight fetuses with "kinky" tails were also seen in the aspirin treated group and one was also found in the group given 2000 ppm PHMB.

No adverse effects in ossification were seen the fetuses from the PHMB treated animals. Over 90% of the aspirin-treated fetal skeletons examined showed an increase in extra ribs. Some "wavy ribs were also seen in this group. The fetuses from the 2000 ppm PHMB group also showed a significant increase in extra ribs.

Treatment with aspirin produced an increase both in incidence and severity of the naturally occurring abnormality hydronephrosis.

Conclusion:

PHMB administered in the diet at levels of 1000 and 2000 ppm maternal toxicity as indicated by reduced weight gain although this was probably related to reduced food intake. Although there may be some suggestion of a slight teratogenic effect at the 2000 ppm dose level (extra ribs), it may be concluded that the NOEL' is 1000 ppm of PHMB in the diet during gestation.

Classification: Core Minimum Data

Section C-12

Appendix to Report No. CTL/P/262 Individual Animal Data. Baquacil SB: A teratology study in the rat by dietary administration.

Classification: Core minimum data as part of Section C-11.

Section C-13

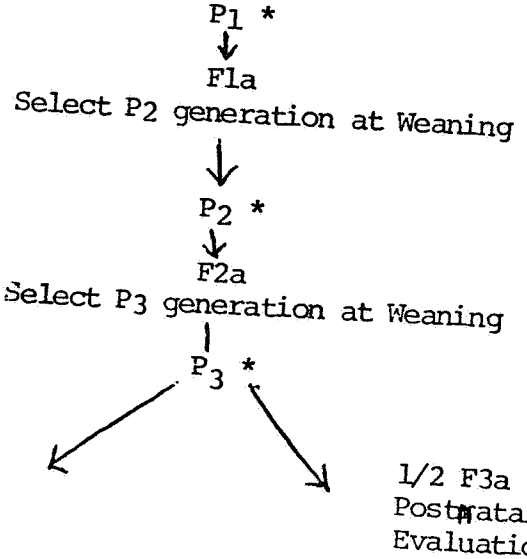
A three-generation reproduction study in rats with 20% PHMB (Hazelton Laboratories America, Inc. final report. January 11, 1977).

Test material: 20% PHMB

Four groups of Charles-River Sprague-Dawley cesarean-delivered albino rats (10 M + 20F per group) received in the diet levels of 0,200, 650 and 1300 ppm through three successive generations according to the following schemata:

Experimental Design

First generation:



*Ten males and twenty females/group

The parents of all three generations were fed the appropriate diets for nine weeks and then subjected to a single mating trial. Offspring from the first two generations (F1a + F2a) were maintained through weaning at which time 10 males + 20 females from each group were selected as parents of the succeeding generation. All P_1 and P_2 offspring that were not selected for subsequent breeding were discarded and approximately one-third of these offspring were necropsied prior to discard. One-half of the third generation offspring (F3a) were taken by cesarean section on Day 19 of gestation and examined for teratogenic effects. The remaining F3a offspring were reared to weaning and then discarded or necropsied (10/sex/group). The following tissues were fixed in 10% neutral buffered formalin and stored for possible future examination:

brain	kidneys	testes with epididymes
pituitary	adrenals	seminal vesicles
eyes	stomach	ovaries
thyroids	pancreas	uterus
lungs	small intestine	bone
heart	large intestine	bone marrow
liver	urinary bladder	unusual lesions
spleen		

Criteria evaluated for compound effect included parental body weight and food consumption data (growth period of P_1, P_2 and P_3 generations) and maternal body weights (during gestation; one-half of P_3 females); parental survival and clinical signs; pregnancy rates and other reproductive indices. Also offspring (litter size was reduced to eight pups by culling after 24 hours of birth) were evaluated for viability, survival, clinical signs, sex ratios, body weights, necropsy findings and teratological findings. Statistical analyses were performed when appropriate by suitable methods.

Results:

No effects attributable to the administration of compound were observed in the evaluation of parental food consumption values, survival rates, clinical findings, pregnancy rates or reproduction data. There were no meaningful differences between body weight data of the control and test parental animals except for slightly decreased body weight gains in the P_3 mid- and high-level males when compared to that of the P_3 control males. This finding is believed to be associated with compound administration.

In addition, evaluation of the various reproductive indices, sex ratios, and body weight data of the fetuses taken by cesarean section and the offspring maintained through meaning revealed no meaningful differences between the control and treated groups. Necropsy of the weanlings did not reveal any compound related gross pathology. No findings indicative of embryo toxicity on treatogenicity were noted in the fetuses taken by cesarean section.

Conclusion:

20% PHMB administered to albino rats through three successive generations at dietary levels of 200, 650, and 1300 ppm did not produce any adverse compound - related effects on reproductive performance for any of the parameters evaluated. Slightly decreased body weight gain occurred in the P3 mid-and high-level male groups and this finding is attributed to compound administration. The NOEL for this reproduction study is 1300 ppm.

Classification: Core Minimum Data

Section C-14

Ninety-Day Oral Toxicity of Antibacterial 9073 - Albino Rats (Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories Report No. IHR/199 August, 1966).

Test Material: Antibacterial 9073 (25% PHMB)

Young adult specific pathogen free (S.P.F.) Wistar rats (25 M + 25 F) received for 90 days in the diet levels of 0, 2500 and 5000 ppm of test material. Food consumption, general observation, body weight, and hematological studies (hemoglobin concentration, packed cell volume (hematocist), white cell counts and differential white cell counts). At the end of the 90-day test period all animals were killed with chloroform and an immediate post mortem examination made. Organ weights were recorded and organ/body weight ratios calculated from a randomly selected five males and five females from each group. The following organs were included: liver, heart, lung, adrenals, kidneys, spleen. Tissues from the remaining animals were fixed in Zenker's fluid, except brains and spinal cords which were fixed in 10% formal saline and examined microscopically. The following were examined: liver, kidney, spleen, heart, lung, adrenals, gonads, thymus, thyroid, pancreas, stomach, duodenum, jejunum , ileum, caecum, colon, salivary gland, mesenteric lymph nodes, spinal cord and brain (cerebrum, cerebellum, and pons).

Results:

All animals survived the 90-day test period. There were no specific adverse effects of the compound. Food consumption was comparable for the test groups and controls. Body weight gain was moderately reduced in males (13.2% less than controls, and females (16.6% less than controls) fed the compound at the highest dietary level (5000 ppm). No abnormalities in hematological parameters were observed. No gross adnormalities were observed. No remarkable change in organ/body weight ratios were detected. Microscopic evaluation revealed that the liver of some females given the compound at a dietary level of 5000 ppm showed an unusual degree of iron pigment both within the liver cells and in Kupffer cells. Although the report states that no iron pigment was seen in animals fed 2500 ppm test material in the diet, the study does not include detailed histopathologucal results of the 2500 ppm animals.

Conclusion

A NOEL cannot be established for this study on the basis of the information unavailable. The laboratory must furnish (a) organ weights recorded (b) detailed histopathological results of the 2500 ppm level animals examined.

Classification

Supplementary Data

a. Organ weights not included

b. No clinical chemistries performed

c. Results of histopathological examination of the 2500 ppm level animals not submitted.

Section C-15

Ninety-Day Oral Toxicity of Antibacterial 9073-Beagle Dogs (Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories Report No. IHR, September 1966).

Test Material: Antibacterial 9073 (25% Aqueous PHMB)

Three groups of Beagle Dogs (4M + 4F per group), 12.4 - 14.6 kg initial BW, received dietary levels of 0,5500, and 11000 ppm of test material and libitum for 90 days. General observation, food consumption, body weight and the following clinico-pathological studies were performed: hemoglobin, packed cell volume (hematocrit), leucocyte count (total) leucocyte count (differential) blood urea, serum alkaline phosphatase, BSP (liver function test) and urine analyses (reaction (PH) specific gravity, glucose, protein, bilirubin, microscopy of centrifuge (deposit). At the end of the test period the animals were killed with an overdose of pentobarbitone administered intravenously. A full post-mortem examination was immediately made and the weight of the following organs were obtained at the time of necropsy: heart, liver, kidneys, adrenals, spleen, thyroid, epididymics, brain and pituitary. Representative pieces of tissues for microscopic examination were taken from the following: brain (cerebrum, cerebellum and ponsl, spinal cord, pituitary, submaxillory gland, thyroid, thymus, heart, lung, aorta, stomach, duodenum, jejunum, ileum, colon, liver, spleen, kidney, bladder, adrenal, ovary and uterus or testis and epididymics, scratic nerve.

Results

Both treated and control animals maintained an excellent condition throughout and no adverse effects were noted. No food consumption data was submitted. Mean body weights were comparable between control and treated animals except for the 11,000 ppm female dogs which gained significantly less total weight than the female dogs. Results of the hematological parameters were unremarkable. Clinical blood chemistries were unremarkable. Results of the BSP liver function test show no difference in retention of BSP attributable to test material. Urine analyses do not appear to be influenced by treatment. Organ/body weight ratios showed no significant variation from the normal as a result of treatment. No gross pathology attributable to test material was detected. Microscopic examination revealed slight hemosiderons in 2 out of 4 males at 11,000 ppm. No other microscopic abnormalities attributable to treatment was present at either dose level.

Conclusion:

The NOEL for this 90 day dog feeding study appears to be 5500 ppm (low dose). The high dose treatment caused a less total weight gain in female dogs and slight hemosiderons in 2 out of 4 male dogs.

Classification: Core Minimum Data

Section C-16

PHMB: 80 Week Skin Painting Study in Mice (Report No. CTL/P/331 Amended).

Test material: 20% PHMB.

Four groups of specific pathogen free (50 M + 50F) (SPF) Aderley Park Mice received dermally 0.3 ml of test material at doses of 0(solvent in ethanol), 0.6 mg (0.2% PHMB in ethanol), 6.0 mg (20% PHMB in ethanol) and 30.0 mg (10.0% PHMB in ethanol) for five days a week for 80 weeks. Clinical investigations included general observation, body weight, food consumption, ophthalmology, gross pathology and histopathology. Any animal which became moribund or distressed due to disease (including tumors) during the course of the experiment was killed and a full post-mortem examination made as described below. Those mice which died spontaneously except for those that were cannibalized, received a full post-mortem examination within 24 hours of death. Where severe autolysis had occurred, only skin and macroscopically-abnormal tissues were fixed for histopathological examination. At termination animals were killed by an overdose of halothane and the following tissues were fixed in formol corrosive: adrenal, cecum, colon, duodenum, epididymis, heart, ileum, jejunum, kidney, liver, lungs, (after perfusion with formol saline) esphoagus, ovary, pancreas, pituitary prostate gland, salivary gland, seminal vesicle, spleen, stomach,

testis, thymus, thyroids trachea, urinary bladder, uterus, voluntary muscle and any abnormal tissue. Lymph nodes (axillary, cervical, inquinal, mesenteric interrenal and mediastinal) were examined during the course of the post-mortem examination, but not preserved unless apparently abnormal. Sciatic nerve, eyes and Harderian glands were submitted in 10% buffered formol saline during the second year. Brain was taken from 50% of the animals in each group killed at 80 weeks and fixed in 10% buffered formol saline. Sections of skin were preserved in Bouin's fixative for histopathological examination. A section of each tissue 5u thick was embedded in paraffin wax and routinely stained with hematoxylin and eosin. All tissues were examined histopathologically. Where appropriate the data were analyzed statistically using Student's "t" test and Chi square.

Results:

Mice receiving the highest dose level (30 mg/day) showed a poorer condition being very thin throughout the experiment. Deaths in both males and female in the highest dose group were slightly higher than in other groups during the first year. This pattern continued throughout the remainder of the study resulting in a high mortality rate (75% in males and females) in the highest dose animals at termination, compared with approximately 30% in the other groups. The highest dose level of PHMB resulted in noticeable irritation to the skin of both males and females immediately after application. Erythema and some clumping of the growing fur was noticed during the first few weeks and after the 4th week, hyperkeratosis became evident especially in males. No differences were apparent between the controls and those mice receiving 0.6 or 6.0 mg PHMB per application. Skin tumors in a total of 6 animals (1M control, 2 male 6.0 mg PHMB, 1 male, 2 female 20 mg PHMB) were observed clinically during the experiment. Although the incidence is low (1% vs 2% and 3%), the effect maybe due to PHMB.

Exophthalmos at the highest dose level increased to greater than 90% in males and females by week 44 and remained at this high level throughout the study. Keratitis (inflammation of the cornea) was seen in many of the affected animals. A few animals from the other groups including controls showed a milder exophthalmos. This reached about 10% (6% for males and 13% for females) incidence at week 80.

A significant reduction ($P \leq 0.05$) in mean body weight was observed for male animals in mid and high dose form weeks 18 and 11, respectively. These differences were maintained throughout the study in the high dose group, but in the mid-dose group, they disappeared in the second year. Mean body weight gains showed a similar pattern.

The females showed a significant reduction (PL0.05) in mean body weight and body weight gain in the high dose after week 56.

There were no overall differences in food consumption for either male or female animals in the treated and control groups at any stage during the course of the experiment. Since there was poorer weight gain for the high dose males, the food utilization for these animals was poorer.

Macroscopic examination revealed the following trends:

Total "growths" (Numbers of animals 50/sex)

Control	0.6 mg PHMB	6.0 mg PHMB	30 mg PHMB
♂ 14 ♀ 8	♂ 9 ♀ 18	♂ 10 ♀ 15	♂ 18 ♀ 25
22	27	24	43

Total "growths" in kidney and livers (No.s of animals)

Control	0.6 PHMB	6.0 mg PHMB	30 mg PHMB
♂ 5 ♀ 2	♂ 4 ♀ 4	♂ 5 ♀ 4	♂ 16 ♀ 7
7	8	9	23

It is apparent from the data that a response in the total number of "growths," also referred to as tumors in the study, in the female mice closely approximates a dose-response relationship. The incidence of "growths" in both sexes also approximates a dose-response relationship and the liver + kidney growths contribute more than 50% of the total for the high dose group. Many of the observations recorded as growths in the macroscopic table are listed in the histopathology table as a moderate to severe form of "Hepatitis" most probably due to the toxic effects of PHMB. There was also a significant increase in the incidence of liver tumors (four in controls and ten in the high dose group), statistically significant only in the case of liver tumors of end otherlial origin (both benign and malignant; two in controls and six in high dose; x^2, 1% level). There was no evidence of such an effect in animals from groups 2 and 3. Also the total number of "neoplasms" was comparable amoung groups as shown below:

Total neoplasms: Number of animals per 50/sex

Control		0.6 mg PHMB		6.0 mg PHMB		30 mg PHMB	
♂	♀	♂	♀	♂	♀	♂	♀
23	15	11	22	22	20	12	18
Total 38		Total 33		Total 42		Total 30	

However, examination of the data shows that all liver tumors represent 4/38 = 10.5% of the total neoplasms of the controls and 10/30 = 33% of the total neoplasms of the high dose group. Liver tumors only represent 3% and 7% of the low and mid-doses respectively. There was evidence that the application of the highest dose of PHMB caused skin irritation. There was epidermal scarring and sometimes inflammation in the superficial regions of the dermis. Occasionally there was ulceration extending to the deeper layers of the dermis at the site of application to skin. Such ulceration was not seen in animals of the low and mid dose levels, and only in one control animal.

Clinical and histological examination of eyes and orbital contents did not show evidence of any pathological abnormality that could account for the bilateral protrusion of eyes that was seen in the high dose animals during the course of the study. Gross and microscopic appearances of thyroids were normal in a large majority of cases.

Conclusion:

PHMB appears to be an hepatic oncogen in the high-dose mice in this study. In addition, the high dose animals demonstrated high mortality, decreased body weight and increased exophthalmos. Also an apparent dose-related/sex related increase in all "growths" of organs microscopically examined in females was observed. Many of the "growths" in the microscopic table were found to be a moderate to severe form of "hepatitis," most probably due to the toxic effects of PHMB. Microscopic evaluation shows liver necrosis occurred in each PHMB treated group to varying degrees in comparison to controls. Therefore there is an effect at each treatment level.

Classification: Core minimum data.

a. The statistically significant incidence of hepatic tumors in the high dose level mice may trigger an RPAR criterion for oncogenicity.

Section C-17

Test experiments concerning the effects of the prolonged oral intake of the product Vantocil IB manufactured by ICI France SA and corresponding to the IB product of our laboratory. (Center for the Exploration of Medical Research, Marseille, France October 19, 1973; Report JMB/601).

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Test Material: Vantocil IB

The animals in each group were treated every day with 100 mg of the product per kg of body weight.

The test group of Wistar rats were as follows:

(a) A group of 20 males of an initial weight of 70 gm, divided into sub-groups of 5 each of these subgroups being placed in a separate cage. This group was introduced into the experiment 5/8/71.

(b) A group of 20 females of initial weight of 70 gm divided into 4 groups of 5. This group was introduced into the experiment on 5/8/71.

(c) Report considers as control groups the numerous previous groups of normal animals (450) studied which have been fed exclusively with the synthetic lacassagne feed.

(d) A group of 10 first generation animals subjected to treatment in April 1972.

(e) A group of 10 second generation animals was subjected to the treatment in January 1973.

The animals were studied from four points of view.

(a) The development of the general condition and the weight (all animals were weighed systematically every fortnight), the clinical behavior and the state of the hair system.

(b) All the animals which died spontaneously or were killed underwent a complete autopsy.

(c) Hematological examination was performed before any animal was killed and a blood sample was taken for electrophoretic examination.

(d) All of the animals which underwent autopsies were the subjects of a complete histological study. The following fragments were taken.

1. At least three fragments of the stomach.
2. Three fragments of the small intestine and large intestine
3. Two fragments of the liver.
4. Two fragments of the spleen.
5. One fregment of the pancreas.
6. Two fragments of the kidneys.
7. Two suprarenal fragments (section of the two suprarenal glands connected by the renal plexus.
8. Two testicular fragments or the ovaries in their entirety.
9. Two fragments of the lungs.
10. Two fragments of the lungs.
11. Two fragments of the heart
12. Four sections from the cervical organs as a whole, including the lymphatic ganglions, the salivary glands, the thyroid glands and the thymus.
13. Three sections of the brain allowing a study of the fronto-parietal cortex, the striated muscles, the thalamus, the hypothalamus, the cerebellum and the sub-thalmo-mesencephalic formations.
14. A section through the sphenoidal bone and surrounding the pituitary fland and the fasser ganglions.
15. A section of the breast-bone and the femoral bone to allow a study of the bone marrow.
16. A section from the mesenteric lymphatic gland.

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Results:

All the animals exhibited a weight curve indentical to that observed in normal animals. The males weighed from 320 to 510 gm. The females weighed from 235, to 390 gm. No findings of any clinically apparent tumors. All animals were killed on September 5, 1973, after 25 months of the study. Macroscopic and histological anatomopathological examination of the males revealed.

1. Absence of sarcomas
2. Absence of hypophysial tumors
3. Absence of auditary tumors
4. Presence of tumor of the testicle in 1 male
5. Chronic bronchopneumopathy processes in 8 males.
6. Pulmonary emphysema in one male
7. A splenic reaction was observed in the rats suffering from chronic bronchopreumopathy.
8. Hypertrophy of the liver in one male.
9. Cystic nephrosis in 3 males.
10. Atrophy of the testicles in 2 rats.
11. Gastro-enteritis in 1 male.
12. Suppurative sinusitis in 4 males.
13. Developed adipose tissue in 2 males.

Summary of the observations made with the female rats.

1. Absence of sarcomas
2. Absence of auditory tumors
3. Absence of hyprophysial tumors
4. Presence of a mammary tumor of the benign adeno-fibroma type in one rat.
5. Chronic bronchopneumopathy processes observed in 2 rats.
6. Pulmonary emphysema in 3 rats.
7. Splenic reactional processes were noted in the rats suffering from chronic bornochopenumopathy.
8. A process of hepatic steatosis in one rat.
9. Cystic nephrous in 3 rats.
10. Hyperplasma of the cortico-suprarenal bodies in 2 rats.

11. Hyperplasia of the uterine mucosa in 5 rats.

12. Hypertrophy of the ovaries in 8 rats.

13. Hyperplasia of the pituitary gland in 5 rats

14. Hyperplasia of the thymus in 2 rats

15. Hyperplasia of the mesenteric ganglions in 3 rats

16. Suppurative sinusitis in 3 rats

17. Well-developed adipose tissue in 4 rats.

18. Hypertrophy of the liver in 1 rat.

Conclusion

Inadequate study which cannot be interpreted from data presented. Historical data not submitted and no controls.

Classification: Inadequate Study

a. Only 20 animals/sex tested. No controls.

b. Only 1 dose level tested not MTD.

c. Unclear in report whether administration is by diet or gavage.

d. Individual animal data on bodyweight, food consumption, mortality, clinical signs, hematology, size of tumors, etc, not submitted.

e. The study of the genetic function and of two generations subjected to experiment is inadequate because only 1 male + 5 females were noted each generation) and report is totally unclear regarding treatment. Only 5M + 5F were treated from each generation.

Section C-18: PHMB: Life-time Feeding Study in the Mouse (Imperial Chemical Industries Limited Central Toxicology Laboratory, Report CTL/P/332 (Amended) - No date

Test material: 20% solution of PHMB containing [redacted]

Groups of 30 male and 60 female Alderley Part strain mice (SPF) were fed diets containing 0, 100, 200 and 1000 ppm PHMB for one week prior to pairing and during mating. Feeding continued for the females throughout pregnancy and lactation. All offspring were weaned at 3 weeks of age. At 5 weeks of age 50 males and 50 females were selected from each group. The offspring were fed the same diets as the parents throughout the experiment. After a further 80 weeks 10 males and 10 females per group were killed for pathological examination.

The experiment was terminated when the overall mortality had reached 80%, 97 weeks after selection of offspring. Parameters investigated were body weight, food consumption and general observations. Any animal which became moribund or distressed due to disease or tumor during the course of the study, those at interim kill and those surviving terminally were killed with halothane and given a full post-morten examination within 24 hours of death. The weights of the following organs were recorded on 10 males and 10 females per group at 80 weeks: liver, kidneys (combined), spleen, heart, lungs (combined), and brain. Sections of the following tissues from all animals were preserved for histopathological examination in formol corrosive: adrenal, cecum, cervix, colon, duodenum, epididymus, heart, ileum, jejunum, kidney, liver, lungs, nesenteric lymph node, mammary gland, esophagus, ovary, pancreas, pituitary, salivary gland, seminal vesicle, spleen, stomach, testis, thymus, thyroid, urinary bladder, uterus, voluntary muscle and any other grossly abnormal tissue. Lymph nodes (para-aortic, axillary, cervical, inguinal, mesenteric, renal and mediastinal) were examined during the course of the post morten examination but not preserved for histopathological examination unless apparently abnormal. Brains and sciatic nerves were taken from animals other those found dead, and preserved in 10% buffered formol saline for histopathological examination. Statistical analyses of data was performed when applicable.

Results

General observations were comparable among groups. After 18 months, the mortalities in all groups for each sex were comparable, although still slightly higher in males than females. The weight gain of the males receiving 1000 ppm PHMB was noticeably higher than controls during the experiment and the weight gain for females receiving 1000 ppm PHMB was significantly lower than controls during the course of the experiment. Male mice receiving 200 or 1000 ppm PHMB consumed less food than controls during the study. The weekly food consumption values were significantly lower for female mice receiving 200 or 1000 ppm diet during the study. There was evidence of increased liver weight in both males and females receiving 1000 ppm PHMB. The mean spleen weight of males receiving 1000 ppm PHMB was significantly higher than control, macroscopic examination of the tissues revealed no differences between groups which could be attributed to the administration of PHMB. The non-neoplastic findings seen in various organs were not treatment related in that pathological changes observed in the treated groups closely resembled those seen in the control animals. There appeared to be a dose-related incidence in neoplasms in the liver as shown below:

Total number of Tumor bearing Animals

Control	100 ppm	200 ppm	1000 ppm
♂ ♀	♂ ♀	♂ ♀	♂ ♀
18 21	16 36	17 25	23 21
Total 39	Total 36	Total 42	Total 44

Neoplasms in Liver

Control	100 ppm	200 ppm	1000 ppm
♂ ♀	♂ ♀	♂ ♀	♂ ♀
2 0	2 0	5 0	6 3
Total 2	Total 2	Total ~~42~~ 5	Total 9

% Liver Neoplasms

Control	100 ppm	200 ppm	1000 ppm
2/39 = 5.1%	2/36 = 5.5%	5/42 = 11.9%	9/44 = 20.9%

Conclusion:

The statistically significant incidence of liver neoplasms ~~significant incidence of liver neoplasms~~ seen in groups treated with PHMB confirms the findings observed in Section C-17. The liver tumors appear to be dose related.

Classification: Core minimum DATA.

a. The statistically significant incidence of liver tumors in mice, which appears also to be dose related, may trigger an ONCOGENIC RPAR criterion.

Section C-19: PHMB: A long term feeding study in rats (Imperial Chemical Industries Limited, Central Toxicology Laboratories, Report No. CTL/P/333 (Amended) August 2, 1977

Test material: 20% solution of PHMB containing [redacted]

Four groups of 60 male and 60 female Adlerley Part Strain (SPF) rats, 6 weeks old, were fed diets containing 0, 200, 1000, and 2000 ppm PHMB. Interim kills were undertaken at 52 weeks (12/sex/group) and 104 weeks (8/sex/group) to provide tissue pathology data at those points. The study was terminated at 124 weeks when 80% mortality had occurred in the control groups and in the experiment overall. Parameters investigated were general observations, bodyweights, food consumption, hematology (hemoglobin hematocrit, total WBC, differential WBC and platelet), Clinical chemistries (plasma alanine transferase, aspartate

trans-aminase, blood urea and blood glucose) and urine analyses. The weights of the following organs were recorded on all animals killed at 52 and 104 weeks: brain, pitiutary, heart, lungs (combined, liver kidneys (combined), spleen, adrenals (combined) and gonads (combined). Animals were killed by inhalation of halothane and the following tissues fixed in formol corrosive were examined microscopically for all animals: adrenal, cecum, colon, duodenum, epididymis, heart, illeum jejunum, kidney, liver, lungs, mesenteric lumph nodes, esophagus, ovary, pancreas, pituitary, prostate gland, salivary gland, seminal vesicle, spleen, stomach, testis, thymus, thyroids, trachea, urinary bladder, uterus and skeletal muscle. Axillary, cervical, inguinal, para-aortic and mediastinal lymph nodes were examined during the course of the necropsy but not preserved for histopathological examination unless apparently abnormal. Brain and spinal cord were submitted in 10% buffered formol saline from 50% of the animals at the interim kills and during the period 104-124 weeks. Statistical Analyses were performed by appropriate methods when applicable.

Results:

General observations, cumulative mortality and accumulative incidence of animals bearing suspected mammary tumors were comparable between control and treatment groups. Reduced mean body weight was dose response related for male rats during the weeks 0-84 for the low-mid- and high-dose level groups in comparison to control rats. Reduced mean body weight was observed during the weeks 0-78 for the mid- and high-dose level female rats but not the low-dose female rats in comparison to controls. Mean body weights for males and females were comparable among control and low and mid-treatment groups after 84-78 weeks respectively, but contained to be reduced in both male and female rats of the high-dose group throughout the study (122 weeks). The food consumption by treated groups during the first ten weeks of the study was reduced compared to the controls. The effect was more apparent for the mid and high dose rats. Between weeks 10-80 the high dose rats ate as well as controls but rats receiving the low and mid doses continued to eat less. After week 80, the fluctuating results made interpretation less clear. Food utilization for high dose rats was reduced. The high dose females showed a slight anemia at 104 weeks. Other hematological parameters were comparable among groups. Clinical chemistries and urinalyses were comparable among groups. Organ weight (52 week) data showed high dose females had increased kidney weight compared to controls. An increase in adrenal weight was apparent for both males and females at 1000 and 2000 ppm PHMB. Necropsy results revealed no findings which were attributable to treatment. The number of tumor bearing animals and the site and incidence of tumors was comparable among treated groups and controls. Microscopic examination revealed an observable increase in the incidence of histocyte conglomerates in the mesenteric lumph nodes in female rats fed 1000 and 2000 ppm PHMB at 52 weeks, 104 weeks, and at termination.

Conclusion:

The administration of PHMB caused body weight reduction in male rats at the low-mid and high-dose levels for the weeks 0-84 of the experiment.

Although the high-dose rats ate amounts of diet comparable to controls and the low and mid dose rats ate less than the controls, the reduced mean body weights for all treated groups are interpreted as being caused by the toxicity of the test material (PHMB) rather than to reduced food intake or palatability effect. Food utilization was reduced in the high dose group only. The NOEL for histopathologic changes is considered to be 200 ppm PHMB. Effects were seen as increased incidence of histiocytosis in the mesenteric lumph nodes at 1000 and 2000 ppm PHMB. Levels up to 2000 ppm in the diet were non-oncogenic to the rats. Although slightly reduced mean body weights was observed in the low dose the NEL for the study is considered to be 200 ppm of PHMB in the diet.

Classification: Core-Minimum DATA.

Section C-20. 26 week Dogs with 20% PHMB. (Hazelton Labs, Project No. 458-123, June 30, 1977).

Test material: 20% PHMB.

Thirty-two healthy adult Beagle dogs 16/sex; approximately 7 to 8 months of age) were divided into four groups (4 sex/group) which received in the diet levels of 0, 500, 1500 and 4500 ppm PHMB for 26 weeks. Criteria evaluated for compound effect included survival, clinical signs, body weight and food consumption values, ophthalmologic findings, BSP, heart rates, electrocardiogram tracings, terminal body weight, urinanalyses, hematological parameters (hematocrit, hemoglobin, RBC, WBC and differential WBC) and clinical chemistries (Fasting blood sugar, BUN, SGPT, AP, total serum bilirubin, SGOT, K^+, Cl^-, Ca^H, Na^+, CO_2, total protein, albumin, serum protein electrophoresis). Following 26 weeks of treatment, the dogs were sacrificed by exsangiunation under surital anesthesia and necropsied.

The following organ weights were taken from each sacrificed dog and the organ/body weight ratios determined: pituitary, thyroid, heart, liver, spleen, kidneys, adrenals, testes with epididymides, prostate, ovaries and uterus.

The above and the following tissues were preserved in 10% neutral buffered formalin: brain, thoracic spinal cord, eyes, mandibular salivary gland, thymus, lung, small intestine (3 levels) large intestine, urinary bladder, mesenteric lumph node, gall bladder, stomach, pancreas, vagina, bone (rib-zinction), bone marrow (femoral plug), skeletal muscle, sciatic nerve and any unusual lesion. All of the formalin preserved tissues from the control and high-dose dogs and the liver, kidneys and pertinent gross lesions from the low and mid dose dogs were stained with hematoxylin and eosin and examined microscopically. Statistical Analyses by the appropriate method were applied.

Results

Body weight losses occurred in the high-dose animals ranging from a mean of 4.8% in the males to 15.9% in the females. One high-dose female lost a total of 41.6% of body weight from initiation of the study through week 26.

This animal and one additional high-dose female were thin in appearance by termination of the study. There was a general decrease in food consumption of the high dose animals during the first week of study, normal food consumption from weeks 2-18, and a decline thereafter through week 26. Evaluation of the clinical laboratory data showed increased SGOT and SGPT beginning week 13 in the high dose animals (males and females). BSP retention values were increased at week 22 for one low-dose female three mid-dose females and for all high-dose males and females. These values were also increased in one low-dose and three high-dose females at week 26. The mean alkaline phosphatase value of the high-dose females was increased at week 4 and scattered instances of individual values increased above normal limits occurred in the mid-and high-dose groups at weeks 13, 22 and 26. The mean electrophoretic alpha 2 fraction value of the high-dose females was decreased at all test intervals and those of the low and mid-dose females at week 13 and 26 suggested a dose-related decrease.

Results of the urinanalyses were not remarkable. Evaluations of the EKG tracings obtained at week 25 showed a slight lowering trend in the P-wave, QRS complex, and the T-wave deflections of the high dose animals which may be related to decreased heart weights in the high dose group. Heart rates were compared among control and test groups.

Evaluation of terminal body weight, organ weights, and organ/body weights ratios showed a treatment-related decrease in the terminal body weights of the high dose animals. Findings considered associated with histopathologically-confirmed tissue alterations included increased in mean relative liver weights and mean absolute and relative kidney weights of the high-dose male and females, the lack of a decrease in the mean liver weight of the high dose males despite the body weight decrease in this group, and markedly elevated absolute and relative liver weights in one mid-dose male.

Treatment-related histopathological changes were noted in sections of the liver and kidneys of the high dose animals and consisted primarily of bile stasis, focal hepatocellular degeneration and necrosis, and focal proxinal tubular nephrosis. Focal hepatocytic degeneration was also seen in one mid-dose male, bile stasis in one mid-dose female and focal tubular regeneration in the kidneys of two mid-dose females.

Conclusion

Administration of 20% PHMB in the diet of male and female beagle dogs for 26 consecutive weeks at dosage levels of 1500 and 4500 ppm produced distinct dose-related hepatotoxicity and nephrosis. Histomorphologic lesions in the mid and high-dose groups consisted primarily of bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal nephrosis. Microscopic examination of liver and kidney sections of low-dose dogs receiving 500 ppm of PHMB in the diet did not reveal any compound related histomorphologic changes in these animals. However, the results of the clinical laboratory studies indicate a compound-related effect on liver function at this level. Elevation of BSP values of one low-dose female is considered predicative of the type of histomorphologic lesions present among the mid and high-dose animals. Therefore there is

not a NEL for this study.

Classification: Core Minimum DATA

Section C-21. Vantocil IB: Absorption and excretion studies in the rat (Report No. CTL/P/16313, March, 1975). 003810

Test Material: Vantocil IB (containing 20% PHMB) has been prepared labeled with C^{14} at an activity of 90 uCi/100 mg. By chromatography this material has been shown to contain at [redacted]

Excretion and Absorption

After dosages of 100 mg/kg BW (4.6 uCi) to 5 male rats, 93% of the radioactivity is excreted in the feces within 5 days; 6% of the radioactivity is found in the urine 0.6% in the bile and 0.2% in the expired air. These findings suggest that Vantocil IB is poorly absorbed from the gut and there is no evidence of enterohepatic recirculation. Vantocil IB appears to be tightly bound to feces. 20% of the bound label can be extracted by dilute acid and chromatography of this material shows a profile similar to Vantocil IB itself. Chromatography of the urinary components shows that they consist almost entirely of high mobility material. Chromatography of Vantocil itself shows materials of low or high mobility and when these fractions are separated and dosed independently to pairs of rats, only 3% of the dose can be recovered when the low mobility material was given, whereas 12% of the dose was given when the material of high mobility was administered. This suggests that the urinary material probably consists of the high mobility components of Vantocil which are absorbed and excreted unchanged. Further studies are required on this aspect.

Tissue Distribution

Groups of three male rats were maintained on a diet containing 100 ppm of labeled Vantocil IB and killed at intervals to determine the accumulation of labeled material in the abdominal fat, liver, kidney and brain during feeding and on their return to normal diet. Concentration in the abdominal fat reached a peak of 1.2 ppm after three weeks and maintained this level for a further two weeks while the animals were in receipt of the diet. After the return to normal diet, concentrations in the abdorminal fat reduced to 0.3 ppm after five weeks, giving a half-life of approximately four weeks.

The concentration in the liver did not exceed 0.6 ppm after five weeks feeding and reduced to undetectable levels within three weeks of the return to normal diet. Comparable concentrations in the kidney and heart were 0.8 and 0.1 ppm maximum concentration. No radioactivity could be detected in the brain. No variations in liver/body weight ratio were detected during this experiment.

Classification: Supplementary DATA

a. Experimental procedures and results are not detailed in this study.

b. Raw data and report do not correspond in tissue study.

Section C-22 : Characterization of the Urinary Polymer-related Material from rats given poly (biguanide-1,5-dihexalnethylene hydrochloride) (Makromol Chem. 177, 2591-2605 (1976), Harold Bratt and David E. Hathway). 003810

Summary

Gastiointestinal absorption in rats of PHMB amounts to only 5.6% of a single oral dose and after subchronic administration of 20 ppm (Cl4) tissue concentrations do not exceed 3 ppm. It has been shown by simple chromatographic and spectroscopic methods that urinary polymer-related material consists of small amounts of PHMB-oligomers, with two cyanoguanidino end groups as well as trace constituents of 3,3-dicyano-1,1'-hexamethylene diguanidine and a compound which is considered to be 1-(6-aminohexyl)-3-cyanoguanidine that is formed during the synthesis of pHMB.

Classification: Core minimum DATA.

R/D init: G.E. Whitmore 5/19/78
gjl
P for GEW 6/28/78

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS
AND DOMESTIC ANIMALS

DANGER:

CORROSIVE. CONCENTRATE CAUSES SKIN AND EYE DAMAGE. HARMFUL OR FATAL IF SWALLOWED.

Do not get in eyes, on skin or on clothing. Avoid breathing vapor or mist. Use with adequate ventilation. Wash thoroughly after handling. Wear goggles or face shield and rubber gloves when handling.

FIRST AID: In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Call a physician.

If swallowed, drink plenty of water or milk. Do not induce vomiting. Call a physician. Wash clothing and decontaminate shoes before reuse.

NOTE TO PHYSICIAN: The potential hazard from ingestion is corrosive action to mucous membranes. Acute systemic toxicity is slight.

ENVIRONMENTAL HAZARDS:

This product is toxic to fish. Do not contaminate water by cleaning of equipment or disposal of wastes. Keep out of lakes, streams, or ponds.

BAQUAC

Swimming Pool Disinfectant

ACCEPTED

14 JUN 1979

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FOR EXPERIMENTAL USE ONL

NOT FOR SALE TO ANY PERSON OTHER THAN A PAR
OR COOPERATOR OF THE EPA-APPROVED EXPERIMENTAL

EPA Exp. Permit No. 10182-EUP-11

EPA Est

DANGER: KEEP OUT OF REACH OF CHILDREN • See s anel
ary statements and statement of First Aid treatment.

ACTIVE INGREDIENT: poly(iminoimidocarbonyliminoimidocarbonyliminohexam
hydrochloride) .....

INERT INGREDIENTS .....

NET WEIGHT 25 KILOGRAMS

MANUFACTURE
ICI AME
Wilmington

Revised 3-22-79

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