45 pages

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

004231

JAN 18 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Baygon Oncogenicity, Mutagenicity and Metabolite Studies
Caswell #508, 508A, 507A, 109A, 495D

TO: Mr. Jay Ellenberger (PM 12)
Registration Division (TS-767)

FROM: Byron T. Backus Byron T. Backus 01/16/85
Toxicology Branch
HED (TS-769)

THROUGH: Robert P. Zendzian, Ph.D., Acting Head
Review Section III
and
William Burnam, Chief
Toxicology Branch

1/17/85

1/17/85

Compound: Baygon, Propoxur, BOQ 5812315, 2-isopropoxy-phenyl-N-methylcarbamate

Registration No. 3125-174 Registrant: Mobay Chemical Corp.

Accession No. 255177

Action Requested:

The Registration Division has asked for an expedited review on a 2-year rat oncogenicity study on the technical material as well as a number of mutagenicity studies on several metabolites.

Background:

The registrant informed the Agency on July 11, 1984 that a 2-year rat feeding study which had been recently completed had shown dose-related toxic effects on the bladder which had included hyperplasia, papillomas and carcinomas. The 2-year rat study was received at the Agency on October 23, 1984, as part of a volume which also included some mutagenicity studies.

According to the Toxicology Branch files, there has been one previous 2-year rat feeding/oncogenicity study reviewed on Baygon. This study was reviewed by K. L. Bailey, and the review was dated 26 October 1977. While a copy of Bailey's review is not available,

his review was cited in a memorandum by Van Ormer (October 19, 1978), which stated: "Since pathology was reported for only five rats per sex per group (in addition to reports of palpated tumors) the study was judged supplementary for oncogenicity."

A 2-year mouse feeding study was reviewed by Zendzian (August 16, 1982). In that review (refer to the attached copy) it was stated that the high-dose (6000 ppm) animals would have been consuming 1191 mg/kg/day for males and 1374 mg/kg/day for the females, and that the reported $LD_{50}$ in rats is of the order of 100 mg/kg; the difference was considered "excessive." There was a request for clarification. Subsequently (Zendzian's memorandum of April 23, 1984, copy attached) it was indicated that this problem had still not been satisfactorily resolved.

New data:

Nine studies on Baygon and its metabolites were received at EPA 10-23-84, and are in Acc. 255177. Studies included one 2-year rat feeding/oncogenicity study on Baygon, 7 mutagenicity studies on Baygon and/or its metabolites, and one metabolism study. Refer to the listing in this memorandum under "Data Evaluation Reports" for individual titles.

Conclusions:

1. The 2-year rat feeding study is Core Minimum Data as an oncogenicity study. Both male and female rats showed a dose-related increased incidence of papillomas and carcinomas of the bladder. All carcinomas and all but one papilloma occurred in high-dose (5000 ppm) rats, with only one male rat at 1000 ppm found to have a bladder papilloma. There was also an dose-related increased incidences of urothelial hyperplasia of the bladder in both 1000 and 5000 ppm rats of both sexes, with almost all rats in the 5000 ppm group showing this finding on autopsy. Females in the 5000 ppm group showed an increased incidence of uterine carcinoma, which may also be an oncogenic effect.

2. Males and females at 5000 ppm showed increased incidences in sciatic nerve neuropathy and muscular atrophy at the final sacrifice. Slight increases in incidence and degree of neuropathy in 1000 ppm rats may have been part of a dose-related trend.

Recommendations:

1. A risk assessment must be developed addressing the exposure-related increased incidences of papillomas and carcinomas of the bladder, as well as the increased incidence of uterine carcinomas. A copy of this report and the memorandum "Exposure Assessment for Propoxur (Baygon)" dated 8 January 1985 from Curt Lunchik will be sent to the Mission Support Staff statisticians in the Toxicology Branch for a risk

assessment on Propoxur.

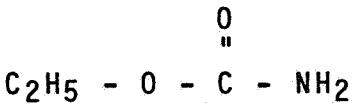
2. No registration actions involving Propoxur (Baygon) should be approved until the risk assessment has been completed.

Discussion:

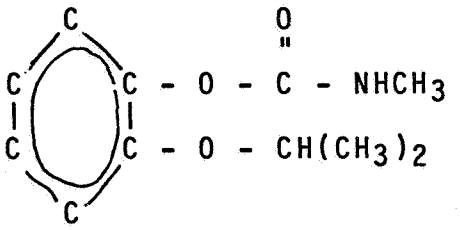
This is the first 2-year rat oncogenicity study on Baygon which is acceptable by current Agency criteria.

While some of the mutagenicity studies showing lack of mutagenic effects were acceptable, there were a number of metabolites which were not tested, and even some that were not identified. Also, the demonstration of a dose-related oncogenic effect in rats takes precedence over the results of the mutagenic studies.

As a speculative possibility regarding a mechanism for oncogenicity from Propoxur, there is a structural similarity to Urethane, a known oncogen:

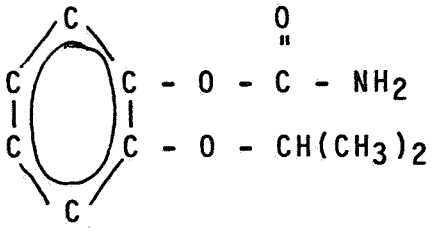


Urethane



Propoxur

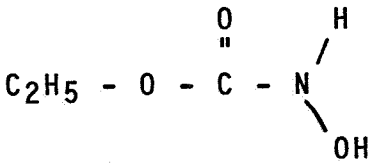
The metabolites of Baygon include 2-isoporpoxyphenyl carbamic acid (refer to OEFZS report no. A050, DER IX of this review). The compound is also designated as THS 1240:



The only mutagenicity studies received for this particular metabolite were those involving rat spleen cells (refer to DER VIII). This particular study would not have detected GC to AT conversions.

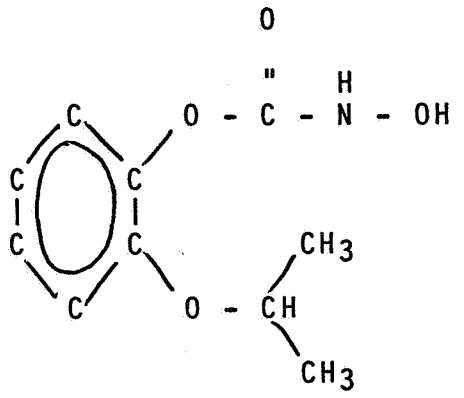
The mechanism by which Urethane acts as an oncogen is believed to involve hydroxylation of the amine group to hydroxyurethan (see Herrion, R. M. "Effects on DNA: Transforming Principle," in Chemical Mutagens, ed. A. Hollaender, Vol. 1, 1971, Plenum Press, NY).

004231

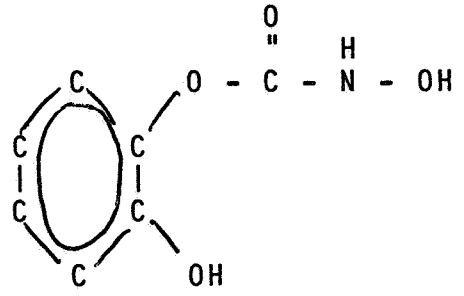


According to Herriott, the mutagenic activity of Hydroxyurethan increases with decreasing pH, and it produces GC-->AT transitions.

By analogy with the above, two possible metabolites of Propoxur that might be more thoroughly investigated for mutagenicity would be the following:



and:



Attachments:

1. Zendzian, R. P. Baygon(R) PP# 2F1244, Review of Two-year Mouse Oncogenicity Study and Teratogenicity Study. Toxicology Branch memorandum dated August 16, 1982.

2. Zendzian, R. P. Baygon; Supplementary Data, Toxicity in Mouse Oncogenicity Study, NOELs Dog and Rat Feed Studies, Metabolism. Toxicology Branch memorandum dated April 23, 1984.

Data Evaluation Reports:

1. Suberg, H. and Löser, E. BOQ 5812315 (common name propoxur) chronic toxicological study with rats (Feeding study over 106

weeks) Report no. 12870, control no. 88501, August 20, 1984.

2. Herbold, B. Isopropoxyphenol test on S. cerevisiae D7 for the induction of mitotic recombination. Report no. 12876, control no. 88506, August 20, 1984.

3. Herbold, B. Brenzcatechin Salmonella/microsome test tto evaluate for potential point mutation. Report no. 12322, control no. 88507; December 20, 1983.

4. Herbold, B. Pol test on E. coli to evaluate for potential DNA damage. Report no. 12497, control no. 88508; February 29, 1984.

5. Herbold, B. THS 2490 Salmonella/microsome test to evaluate for point mutation. Report no. 12529, control no. 88509, March 6, 1984.

6. Herbold, B. THS 1241b Salmonella/microsome test to evaluate for potential point mutation. Report no. 12795; control no. 88510; September 7, 1984.

7. Herbold, B. Isopropoxyphenol Salmonella/microsome test to evaluate for point mutation. Report no. 12321; control no. 88581; December 20, 1983.

8. Klein, W., Kocsis, F., Lippl, K., Schwarzinger, B. and Bornatowicz, N. Effect of an active ingredient and three metabolites on the DNA metabolism. OEFZS report no. A050, BL-453/84, control no. 88582; March 1984.

9. Eben, A. and Karl, W. Studies on the biotransformation of Propoxur in the rat. Report no. 12866, control no. 88584; August 17, 1984.

Data Evaluation Report (I)

004231

Compound:

2-isopropoxy-phenyl-N-methylcarbamate, BOQ 5812315, Propoxur, Baygon

Study type:

2-year rat feeding and oncogenicity

Citation:

Suberg, H. and Löser, E. BOQ 5812315 (common name propoxur) chronic toxicological study with rats (Feeding study over 106 weeks). Report No. 12870, dated August 20, 1984. Studies conducted at Wuppertal-Elberfeld. Received at EPA 10-23-84; in Acc. 255177.

The report also contains the following:

Glaister, J.R. BOQ 5812315: 2 year carcinogenicity/chronic toxicity study in the rat histopathology report. HLE Report no. 3463-262/32, dated June 13, 1984. Work done at Hazleton Laboratories Europe Ltd, in England.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Byron T. Backus 01-15-85

1/17/85

Approved by:

Robert Zendzian, Ph.D.
Acting Section Head
Toxicology Branch

Core Classification: Minimum (as an oncogenicity study)
Supplementary (as a 2-year feeding study)

Conclusions:

1. The study has demonstrated that the test material (or one of its metabolites) is an oncogen. Bladder carcinomas were observed at termination in 8/49 male and 5/48 female rats at the highest exposure level (5000 ppm). More than half (25/49 males, 28/48 females) the rats at this exposure level had papillomas of the bladder, and almost all had epithelial hyperplasia of the bladder. Epithelial hyperplasia of the bladder was also present in 10/50 males and 5/49 females at the 1000 ppm exposure level, and one male in this group with hyperplasia also had a bladder papilloma.

2. Although not statistically significant, the increased incidence of uterine carcinoma in 5000 ppm group females as compared to controls (8/48 vs. 3/49) may also be an exposure-related oncogenic effect.

3. A NOEL for the feeding study was not established for males, as mean body weights for males of the 200 ppm group were lower than those of controls throughout the study (and were significantly lower during the period from 2 to 20 weeks). This effect was part of an exposure-related trend. A NOEL

was established for females at 200 ppm.

004231

4. In both males and females at 1000 ppm there was a slight increase in incidence and degree of neuropathy of the sciatic nerve at the end of the study above that seen in controls. There was 1-5% reduction (often significant at $p \leq 0.05$) in mean body weight relative to that of controls during the study, and there was a greater incidence of epithelial hyperplasia of the bladder.

5. At 5000 ppm there were significant (generally at $p \leq 0.01$) depressions in body weights for both males and females, along with significantly less food consumption throughout the study. At termination, there were increases in incidence and severity of neuropathy and muscular atrophy. Mean plasma ChE activities of females were lower (significant at 13, 26 and 52 weeks) than those of controls. Females had significantly higher concentrations of blood urea at 6, 12 and 18 months. Other effects (increased incidence of splenic atrophy, 4/10 males showing increased thromboplastin times at 24 months) probably indicate that this group was somewhat less healthier than controls and rats at 200 and 1000 ppm.

Materials:

SPF rats, strain BOR:WISW (SPF Cpb) from Winkelmann, Borchen.

Test material, designated as BOQ 5812315 (a mixed batch from batches numbered 234001222-234001226), with 99.4% active ingredient.

Procedure:

Sixty rats of each sex were randomly assigned to each of four groups, which received 0, 200, 1000 or 5000 ppm of the test material mixed in with the diet for the next 106 weeks. Rats were caged singly.

During the study diet mixtures were analyzed. According to a report at the end (pages 845-846) of the study mixtures containing 200, 1000 and 5000 ppm of the test compound were analyzed every 3 months using HPLC. There was also a test for homogeneity of the test compound in the 200 and 5000 ppm diets on one occasion, and a single 10-day storage stability test.

Rats were inspected twice daily (once on weekends and holidays). Changes or signs were recorded on a weekly basis. Rats were weighed weekly (except from week 27 to 61 when this was done biweekly). Weekly food consumption was determined by weighing the uneaten food.

Clinical laboratory examinations were carried out on blood and urine from 10 males and 10 females of each dose group at 6, 12, 18 and 24 months. Insofar as was possible the same animals were used on each of these dates. Measurements were made on the following hematology parameters:

| | | |
|---|---|---|
| Erythrocyte count | MCV value | MCHC |
| Leucocyte count | Haemoglobin | Haematocrit |
| Thrombocyte count | MCH | Differential count (Wright's method) |

Thromboplastin time was determined at the end of the study.

004231

The following clinical chemistry measurements were made:

| | |
|---|---|
| Alkaline Phosphatase (ALP) | Glutamate Pyruvate Transaminase (GPT) |
| Glutamate Oxalacetate Transaminase (GOT) | Cholinesterases (RBC and Plasma)* |

*Cholinesterase measurements were also made at 13 weeks.

The amounts of the following were measured in the plasma:

| | | |
|---|---|---|
| Creatinine | Blood sugar | Bilirubin |
| Urea | Cholesterol | Total protein |

Serum concentrations of Na, K and Ca were determined.

The urine was examined for glucose, blood, protein, pH, ketone bodies, bilirubin, urobilinogen and protein. The sediment was examined microscopically after centrifugation.

Rats which died or were sacrificed in a moribund condition during the study study were dissected and grossly appraised. Organs and tissues which appeared still evaluable were fixed in 10% formaldehyde.

At 52 weeks 10 males and 10 females randomly selected from each group were anesthetized with diethylether and sacrificed by exsanguination. They were then dissected and grossly examined. At 106 weeks all survivors were similarly sacrificed.

The following organs and tissues were fixed in 10% formaldehyde solution:

| | |
|---|---|
| Aorta | Stomach |
| Eyes | *Spleen |
| Intestine (duodenum, jejunum, ileum colon, "partly" caecum and rectum) | *Adrenals |
| Femur en bloc with skeletal musculature and n. ischiadicus | *Kidneys |
| Brain | *Ovaries |
| Urinary bladder | Pancreas |
| *Heart | Prostate |
| *Testicles | Spinal marrow |
| Pituitary | Seminal vesicle |
| Salivary glands | Sternum |
| *Liver | Thyroids |
| *Lung | Esophagus and trachea en bloc |
| Lymph nodes (mesenteric and "non-mesenteric") | Thymus (when present) |
| | Uterus |
| | All grossly apparent "alterations" |

Organs marked with an asterisk were weighed.

Tissue specimens from all rats were processed to paraffin blocks, sectioned at a nominal 5 um and stained with hematoxylin and eosin. Because bladder effects were suspected additional slides were prepared from this organ when there was sufficient additional fixed tissue available.

Statistics:

Arithmetic group means, standard deviations, and upper and lower confidence limits for 1 - alpha = 95% and 1 - alpha = 99% were calculated.

Results:

Analysis of the diet for test material indicated concentrations ranging from 82 to 129% of the theoretical value (p. 845). Homogeneity was apparently tested on only a single occasion (but it is not stated when). Results (p. 846) indicate on this one occasion the 200 and 5000 ppm diets were homogeneous, but it is not certain how samples were selected other than "by means of random numbers." Another series of analysis (p. 846) indicate the compound was "stable in the diet for 10 days" at levels of 200 and 5000 ppm.

There were no exposure-related differences in mortality between groups. No signs of cholinesterase inhibition were observed.

Mean body weights for females at 200 ppm were essentially the same as those of controls. Mean body weights for males at 200 ppm were depressed relative to controls thoughout the study, but this was statistically significant only from week 2 through 20, and again briefly at week 33 through 35. Mean body weights for both males and females at 1000 ppm were generally lower than control values, and differences were frequently significant at $p \leq 0.05$. For rats at 5000 ppm mean body weights were significantly ($p \leq 0.01$) lower than control values during the entire study.

Mean food consumption, expressed on a grams/week/body weight basis, was obviously depressed in both male and female rats at 5000 ppm throughout the study (the table running from page 134 through 142 does not give a statistical analysis). Mean food consumption for rats at 1000 ppm usually (but not invariably) was less than the corresponding control value. Females at 200 ppm showed no difference from controls, but mean food consumption for males at 200 ppm was generally less than control values; however, this was not dose-related as on more than 50 occasions mean food consumption for males at 200 ppm was less than that for males at 1000 ppm.

Hematology:

Mean HGB values for 5000 ppm females were significantly greater ($p < 0.01$) than corresponding control values at 6, 12 and 18 months; for males at 5000 ppm this occurred only at 6 months. Males at 5000 ppm showed a slight elevation in HGB relative to controls at 18 months, but this was not statistically significant at $p \leq 0.05$.

Mean thromboplastin time (measured only at 24 months) was greater in males at 5000 ppm than in controls (as calculated from individual data on pages 335-336); although probably significant the data were not analyzed statistically. Four of 10 males at 5000 ppm had thromboplastin times greater than 17 seconds, greater

than any male from any of the groups. Among females, one rat at 1000 and one at 5000 ppm (data on pages 339-340) were the only subjects showing thromboplastin times greater than 17 seconds, but differences between the means of the different groups were probably not statistically significant.

In the blood differential counts, males at 5000 ppm consistently showed a slightly higher (but not significantly different) mean eosinophile count relative to controls and the other 2 groups. In females at 5000 ppm the mean eosinophile count was significantly greater than that for controls at 6 months ($p < 0.05$) and at 24 months ($p < 0.01$). However, there are no exceptionally high values in the individual data (p. 313-338) for any of the rats at 5000 ppm.

Variations in the other haematology measurements were incidental, with no consistent trend or evidence for an exposure-related pattern apparent.

Clinical chemistry:

There was a tendency for high-exposure rats to show less mean glutamic oxalacetic transaminase (GOT) activity than controls. The following is from the tables on pages 20-23:

| Males | 6 months | 12 months | 18 months | 24 months |
|---|---|---|---|---|
| 200 ppm | 0 | 0 | 0 | - |
| 1000 ppm | --- | 0 | 0 | --- |
| 5000 ppm | --- | --- | -- | --- |
| **Females** | | | | |
| 200 ppm | - | + | + | 0 |
| 1000 ppm | -- | + | - | 0 |
| 5000 ppm | --- | 0 | -- | - |

+ higher than controls, but no statistically significant difference
0 essentially the same activity as controls
- lower activity than controls, but no statistically significant differance
-- lower activity than controls, $p < 0.05$
--- lower activity than controls, $p \leq 0.01$

Females at 1000 ppm had higher ($p < 0.01$) mean urea concentrations at 6 months. Females at 5000 ppm had significantly higher mean urea concentrations at 6, 12 and 18 months. However, at 24 months mean urea concentrations were essentially the same for controls and 5000 ppm female rats (Tables 7b, 8b, 9b and 10b, pages 21-24).

Males at 5000 ppm had higher mean cholesterol levels at 6 and 12 months ($p \leq 0.01$) and again at 18 months ($p < 0.05$). Mean cholesterol levels were about the same for controls and 5000 ppm male rats at 24 months.

Mean plasma ChE activities of 5000 ppm females were consistently lower (significant at $p < 0.05$ at weeks 13, 26 and 78) than control values. From the data in table 11, p. 25, the mean plasma ChE activities (with controls = 1.000) for the different female groups were:

004231

| | week 13 | week 26 | week 52 | week 78 | week 104 |
|---|---|---|---|---|---|
| 200 ppm | 1.377 | 1.275 | 1.345 | 1.369 | 1.364 |
| 1000 ppm | 0.968 | 1.010 | 0.995 | 1.150 | 1.170 |
| 5000 ppm | 0.805 | 0.785 | 0.850 | 0.856 | 0.949 |

The consistency of these values for each group is striking.

Mean RBC ChE activities for males at 5000 ppm were significantly ($p \leq 0.01$) less than those of controls at weeks 26 and 104; females at 5000 ppm had significantly ($p \leq 0.01$) less mean RBC ChE activity than controls at 13 weeks. However, no consistent trends were evident on other occasions.

Although the report states (p. 27) that there was a dose-related statistically significant variation in Na ion in males at 18 months, this situation (with a trend of increasing Na ion concentration with exposure level) was observed only on this one occasion. Females at 5000 ppm showed significant reductions in Ca at 18 and 24 months.

Histopathology:

Mean organ weights of the 5000 ppm rats tended to be significantly less than those of controls. However, rats at 5000 ppm had mean body weights which were significantly less than those of controls throughout the study. Mean organ to body weight ratios from 5000 ppm rats were usually significantly (often at $p \leq 0.01$) greater than those of controls.

Although not statistically significant, there was a greater incidence of splenic atrophy in rats at 5000 ppm than in controls and animals at 200 and 1000 ppm; from table 3, p. 533:

| | 0 ppm | | 200 ppm | | 1000 ppm | | 5000 ppm | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Splenic atrophy | 3/49 | 3/49 | 2/50 | 1/46 | 1/50 | 2/49 | 7/49 | 5/48 |

Splenic atrophy was a finding in 7/13 males and 4/12 females of the high dose group which died "sporadically" and were examined. Corresponding values for the controls were 3/12 males and 2/11 females.

In rats at 5000 ppm there was a definite increase in incidence and degree of both neuropathy of the sciatic nerve and muscular atrophy. Although not statistically significant, the slightly greater incidence of neuropathy in 1000 ppm rats appears to be part of a dose-related trend. From table 22, p. 33 (also table 8, p. 547) for rats which were terminated at the end of the study:

| | 0 ppm | | 200 ppm | | 1000 ppm | | 5000 ppm | |
|---|---|---|---|---|---|---|---|---|
| neuropathy, n. ischiadicus | M | F | M | F | M | F | M | F |
| not examined: | 0 | 0 | 0 | 1 | 3 | 2 | 0 | 1 |
| not observed: | 13 | 13 | 14 | 13 | 12 | 11 | 1 | 5 |
| very slight: | 14 | 17 | 21 | 14 | 17 | 12 | 9 | 3 |
| slight: | 8 | 7 | 9 | 11 | 6 | 13 | 13 | 17 |
| medium: | 2 | 1 | 0 | 1 | 3 | 2 | 10 | 9 |
| severe: | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |

004231

| muscular atrophy | 0 ppm M | 0 ppm F | 200 ppm M | 200 ppm F | 1000 ppm M | 1000 ppm F | 5000 ppm M | 5000 ppm F |
|---|---|---|---|---|---|---|---|---|
| not observed: | 34 | 37 | 43 | 40 | 39 | 40 | 17 | 26 |
| very slight: | 3 | 0 | 1 | 0 | 0 | 0 | 8 | 6 |
| slight: | 0 | 1 | 0 | 0 | 0 | 0 | 7 | 4 |
| medium: | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 0 |
| severe: | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| number animals examined | 37 | 38 | 44 | 40 | 41 | 40 | 36 | 36 |

The values above differ slightly from those in table 3, p. 532:

| | 0 ppm M | 0 ppm F | 200 ppm M | 200 ppm F | 1000 ppm M | 1000 ppm F | 5000 ppm M | 5000 ppm F |
|---|---|---|---|---|---|---|---|---|
| Sciatic n. neuropathy p. 33 cumulative total any observed | 24 | 25 | 30 | 26 | 26 | 27 | 35 | 30 |
| Sciatic n. neuropathy p. 532 | 25 | 25 | 32 | 27 | 30 | 27 | 38 | 34 |

The differences are due to findings in some of the rats which died before final sacrifice at 106 weeks.

By assigning values to the degree of sciatic neuropathy (0 = not observed, 1 = very slight, 2 = slight, 3 = medium, and 4 = severe) and using the data in table 22, p. 33 a quantitative mean for each group can be obtained:

Males

| | | 0 ppm | 200 ppm | 1000 ppm | 5000 ppm |
|---|---|---|---|---|---|
| not observed | 0 | 0 = 13 x 0 | 0 = 14 x 0 | 0 = 12 x 0 | 0 = 1 x 0 |
| very slight | 1 | 14 = 14 x 1 | 21 = 21 x 1 | 17 = 17 x 1 | 9 = 9 x 1 |
| slight | 2 | 16 = 8 x 2 | 18 = 9 x 2 | 12 = 6 x 2 | 26 = 13 x 2 |
| medium | 3 | 6 = 2 x 3 | 0 = 0 x 3 | 9 = 3 x 3 | 30 = 10 x 3 |
| severe | 4 | 0 = 0 x 4 | 0 = 0 x 4 | 0 = 0 x 4 | 12 = 3 x 4 |
| Group Mean | | 36/37 = 0.97 | 39/44 = 0.89 | 38/38 = 1.00 | 77/36 = 2.14 |

Females

| | | 0 ppm | 200 ppm | 1000 ppm | 5000 ppm |
|---|---|---|---|---|---|
| not observed | 0 | 0 = 13 x 0 | 0 = 13 x 0 | 0 = 11 x 0 | 0 = 5 x 0 |
| very slight | 1 | 17 = 17 x 1 | 14 = 14 x 1 | 12 = 12 x 1 | 3 = 3 x 1 |
| slight | 2 | 14 = 7 x 2 | 22 = 11 x 2 | 26 = 13 x 2 | 34 = 17 x 2 |
| medium | 3 | 3 = 1 x 3 | 3 = 1 x 3 | 6 = 2 x 3 | 27 = 9 x 3 |
| severe | 4 | 0 = 0 x 4 | 0 = 0 x 4 | 0 = 0 x 4 | 4 = 1 x 4 |
| Group Mean | | 34/38 = 0.89 | 39/39 = 1.00 | 44/38 = 1.16 | 68/35 = 1.94 |

Six males (409, 412, 413, 417, 419 and 420) in the 5000 ppm group had atrophied musculature in the rear extremities (refer to the table of gross pathological findings, p. 503-505). The only other rat in the study reported in gross pathological findings as having atrophied musculature was #86, a control female. In the individual animal pathology reports (p. 770-776) the muscle findings for these rats are:

409. Atrophy: minimal multifocal.
412. Atrophy: slight multifocal.
413. Atrophy: minimal focal
417. Lesion: atrophy described at necropsy not apparent histologically
419. Atrophy: moderate multifocal
420. Atrophy: slight multifocal

004231

It is noteworthy that these 5 cases occurred in a relatively short subject numerical sequence.

Bladder effects:

At the interim (one year) sacrifice 4/10 males and 2/10 females at 1000 ppm and all 10 males and 10 females at 5000 ppm had urethelial hyperplasia of the bladder. One male at 5000 ppm had a papilloma of the bladder.

For animals dying during the study or sacrificed at termination incidences of urothelial hyperplasia of the bladder were (from table 3, p. 529):

| | controls | 200 ppm | 1000 ppm | 5000 ppm |
|---|---|---|---|---|
| Males | 1/49 | 1/50 | 10/50 | 44/49 |
| Females | 0/49 | 0/46 | 5/49 | 48/48 |

The degree and extent of urothelial hyperplasia of the bladder were also correlated with exposure level. Males at 0 and 200 ppm (rats #24 and #158 respectively) had "minimal focal" hyperplasia. In some rats at 1000 ppm the finding is reported as "diffuse" or "multifocal," while rats at 5000 ppm tended to have a diffuse hyperplasia which was either "moderate" or "marked."

Almost all papillomas of the bladder occurred in males and females at 5000 ppm with one 1000 ppm male also with this finding; all rats with bladder papillomas also had urothelial hyperplasia. From table 4, p. 535:

| | controls | 200 ppm | 1000 ppm | 5000 ppm |
|---|---|---|---|---|
| Males | 0/49 | 0/50 | 1/50 | 25/49 |
| Females | 0/49 | 0/46 | 0/49 | 28/48 |

Carcinoma of the bladder occurred only in male and female rats of the 5000 ppm exposure group; from table 4, p. 535:

| | controls | 200 ppm | 1000 ppm | 5000 ppm |
|---|---|---|---|---|
| Males | 0/49 | 0/50 | 0/50 | 8/49 |
| Females | 0/49 | 0/46 | 0/49 | 5/48 |

Associations of urothelial hyperplasia, papillomas and carcinomas of the bladders are given in table 26b (p. 40):

| | 0 ppm | | 200 ppm | | 1000 ppm | | 5000 ppm | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| epithelium alone | 1 | 0 | 1 | 0 | 9 | 5 | 13 | 15 |
| epithelium + papilloma | 0 | 0 | 0 | 0 | 1 | 0 | 25 | 28 |
| hyperplasia of bladder epithelium + carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 5 |
| papilloma + carcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| bladder carcinoma alone | 0 | | 0 | 0 | 0 | 0 | 2 | 0 |

In two cases (animals 362, 369) among males at 5000 ppm bladder carcinoma, but no hyperplasia of the bladder epithelium, was reported. However, in both rats the carcinoma was probably of urothelial origin (animal 362: the carcinoma is described as "a large urothelial proliferation extending into the lumen of the bladder..." In animal 369 the carcinoma was "a large exophytic proliferation of plump urothelial cells focally invading the base of the stalk").

The individual animal pathology data descriptions of the bladder carcinomas indicate that most (at least 11 out of 13) were probably derived from urothelial tissue.

Uterine carcinoma:

The incidence of carcinoma of the uterus is given below (from table 7, p. 545):

| | 0 ppm | 200 ppm | 1000 ppm | 5000 ppm |
|---|---|---|---|---|
| Non-metastatic: | 2 | 2 | 1 | 3 |
| Metastatic: | 1 | 2 | 2 | 5 |
| Total carcinomas: | 3 | 4 | 3 | 8 |

There was a increased incidence of uterine carcinoma in females at the highest exposure level, although this increase was apparently not statistically significant. Nevertheless, there was an exposure-related trend, and there was a definite tendency for these carcinomas to develop earlier and/or to grow more rapidly in females at the highest-dose level than in the other groups.

Discussion:

The study demonstrates oncogenic effects occurring in an exposure-related pattern with the test material. Oncogenic effects were papillomas and carcinomas of the bladder in both males and females. The increased incidence (although not statistically significant it was more than twice that observed in controls) of uterine carcinoma in 5000 ppm females also may have been an oncogenic effect. There may be a similarity in the uterine and bladder environments that makes these organs more susceptible than others to the development of carcinomas on exposure to the test material or one of its metabolites.

On p. 41 a statement is made that: "...the increased incidence of hyperplasia of the bladder, noted at the interim autopsy after one year from the dose of 1000 ppm onwards, was not observed at end of study, but this condition occurred...in the 5000 ppm dose group." However, a number of rats in the 1000 ppm group showed exposure-related urothelial hyperplasia at the end of the study. The registrant then appears to attempt an argument that the bladder carcinomas were not necessarily related to the papillomas and/or epithelial hyperplasia of the bladder, as no joint occurrences were found of papillomas and carcinomas, and there were two carcinomas of the bladder in the 5000 ppm group

in which no epithelial hyperplasia of the bladder was found. This section was apparently written at Wuppertal-Elberfeld, rather than at Hazleton Laboratories of Europe, where the actual histologic observations were made.

Although the report states that there were no associations of papillomas with carcinomas, individual animal pathology data suggest this is somewhat equivocal. In animal 375 the carcinoma was "a large partly papillary partly solid exophytic proliferation of urothelial cells..." In animal 412 (not considered to have bladder carcinoma) a papilloma was a "small endophytic proliferation of nests of small cells extending into submucosa. Equivocally early carcinoma." In animal 457 the carcinoma was "a large exophytic papillary growth."

With respect to the occurrence of bladder carcinomas without urothelial hyperplasia, it is noted on p. 44: "In the case of the two bladder carcinomas in the 5000 ppm group males, where no hyperplasia of the bladder epithelium was recorded, it cannot be ruled out that locally hyperplastic areas existed in in the bladder epithelium which were, however, not contained in the histological plane of section, and which therefore were not recorded."

Among non-oncogenic effects, all groups of treated males showed depressed body weights with respect to controls, and the degree of depression was correlated with the degree of exposure to the test material. A NOEL was not observed with respect to body weight depression, although this effect was statistically significant for the 200 ppm males only during the period from weeks 2 through 20 (and again briefly during weeks 33-35). Among females, only the rats at 1000 and 5000 ppm showed depressed body weights with respect to control values.

The most serious non-oncogenic effects included a neuropathy and probably associated muscular atrophy, both definitely present at 5000 ppm. A slight (not statistically significant) increase in severity of neuropathy appears to be present at 1000 ppm, and it is possible that the "medium" muscular atrophy observed in two males at 1000 ppm was also due to exposure to the test material.

The greater incidence of splenic atrophy at 5000 ppm, even though not statistically significant, may be an effect. The initial interpretation was that its occurrence was simply an indication that the high-dose rats were in somewhat poorer health than rats in the other groups, but the report (submitted along with this study) that two of the metabolites of Baygon have a suppressive effect on programmed DNA synthesis in rat spleen cells suggersts a possibly more direct cause and effect relationship.

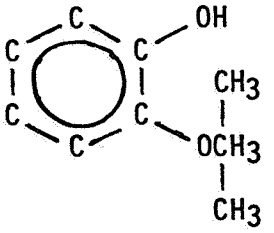
The situation with respect to most of the haematology and clinical chemistry values also probably indicates that the animals at 5000 ppm tended to be in poorer health than those of other groups. This would explain the greater mean thromboplastin time for males at 5000 ppm at 24 months, and the tendency for both males and females in this group to have less mean glutamic oxalacetic transaminase (GOT) activity.

However, the plasma ChE depression in 5000 ppm females may have been a direct effect of exposure to the test material.

Data Evaluation Report (II)

Compound:

2-Isopropoxyphenol (VK32-806; a metabolite of Propoxur)



Citation:

Herbold, B. Isopropoxyphenol test on S. cerevisiae D7 for the induction of mitotic recombination. Report no. 12876, control no. 88506, 08-20-84. Study conducted at the Bayer AG Institute of Toxicology. Received at EPA 10-23-84; in Acc.255177.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Approved by:

Robert Zendzian, Ph.D.
Acting Section Head, Section III
Toxicology Branch

Core Classification: Acceptable

Conclusions:

1. The test material in concentrations of up to 3000 ug/ml did not induce gene conversion, either with or without S-9 activation, in Saccharomyces cerevisiae D7. Without S-9 mix, there was no increased incidence in crossing-over at up to 3000 ug/ml. With S-9 mix, there was no increased incidence of mitotic crossing-over at up to 1000 ug/ml, but it cannot be stated whether or not there was increased incidence of mitotic crossing-over at and above 1500 ug/ml.

2. Above 2500 ug/ml the test material is cytotoxic. There may be cytotoxicity between 1000 and 2500 ug/ml, but this is equivocal.

Materials:

2-isopropoxyphenol (VK32-806), a metabolic product of propoxur.

4-nitrochinoline-N-oxide (4-NQO; Fluka), batch 212295185, a known mutagen.

Endoxan (Asta), batch 092316, active ingredient cyclophosphamide, a bifunctionally alkylating agent and a known promutagen.

Saccharomyces cerevisiae D7, with the genotype:

$$\frac{\text{ade2-40, trp5-12, ilvI-92}}{\text{ade2-119, trp5-27, ilvI-92}}$$

Only if gene conversion (or gene mutation, a rarer event) has occurred will the organism grow on a tryptophane-free medium.

Mitotic crossing-over is detected by means of the marker alleles ade2-40 and ade2-119. The heterozygote is capable of growth without adenine, and colonies are white. The ade2-40 homozygote is adenine-dependent, and the colonies are red. Ade2-119 has a slight adenine dependency, and the colonies are pink. If segregation takes place on a solid medium a colony is formed with red and pink sectors, the red sector usually being smaller than the pink.

S-9 mix, produced from the livers of at least 6 adult male Sprague-Dawley rats. For enzyme induction the animals were given a single IP injection of aroclor 1254 (dose of 500 mg/kg body weight) dissolved in peanut oil five days before preparation.

Procedure:

One ml of yeast suspension from a permanent culture was placed in 9 mls of medium and incubated for 5 hrs at 28° C and 150 rpm (presumably a shaker table of some sort). The culture was then brought to a cell density of approximately $10^8$ cells/ml with medium, and was used immediately for the test.

0.5 ml of yeast suspension was incubated for 16 hrs at 37° C and 150 rpm in a mixture of medium, buffer, test solution, and, where appropriate, S-9 mix, with a total volume of 5 mls.

To test for mitotic gene conversion, 0.1 ml suspension from each tube was plated onto 5 tryptophane-free plates of nutrient broth.

To test for mitotic crossing-over and toxicity "suitable" solutions were prepared from each tube and plated onto ten full medium plates, so that approximately 200 colonies would develop per plate.

In the first series, concentrations of isopropoxyphenol tested were 0 (negative control), 625, 1250, 2500, 5000 and 10000 ug/ml, with positive control concentrations of 60 ug/ml Cyclophosphamid with S-9 mix and 75 ng/ml 4-NQO without S-9 mix.

In the second series, concentrations of isopropoxyphenol were 0, 187.5, 375, 750, 1500 and 3000 ug/ml, with positive control concentrations the same as in the first series. Again, testing was with and without S-9 mix.

In the last series testing for mitotic crossing-over and gene conversion was at 0, 185.9, 260.3, 364.9, 510.2, 714.3 and 1000 ug/ml isopropoxyphenol or 75 ng/ml 4-NQO without S-9 mix, and at 0, 510.2, 714.3, 1000, 1400, 1960 and 2744 ug/ml or 60 ug/ml Cyclophosphamid with S-9 mix.

Results:

Mitotic Crossing-Over

(Mean number of colonies/plate with red and/or pink areas)

| Concentration of Isopropoxyphenol in ug/ml: | I w/o S-9 | I with S-9 | II w/o S-9 | II with S-9 | III w/o S-9 | III with S-9 |
|---|---|---|---|---|---|---|
| 0 | 0 | 0.3 | 0.5 | 0.5 | 0.1 | 0.2 |
| 185.9 | - | - | - | - | 0 | - |
| 187.5 | - | - | 0.4 | 0.7 | - | - |
| 260.3 | - | - | - | - | 0.2 | - |
| 364.4 | - | - | - | - | 0.1 | - |
| 375 | - | - | 0.8 | 0.1 | - | - |
| 510.2 | - | - | - | - | 0.2 | 0 |
| 625 | 0.2 | 0 | - | - | - | - |
| 714.3 | - | - | - | - | 0.2 | 0.3 |
| 750 | - | - | 0.3 | 0.2 | - | - |
| 1,000 | - | - | - | - | 0 | 0 |
| 1,250 | 0.2 | 0.5 | - | - | - | - |
| 1,400 | - | - | - | - | - | 0 |
| 1,500 | - | - | 0.6 | 1.2 | - | - |
| 1,960 | - | - | - | - | - | 0.2 |
| 2,500 | 0 | 0 | - | - | - | - |
| 2,744 | - | - | - | - | - | 0.2 |
| 3,000 | - | - | 0.3 | N.E. | - | - |
| 5,000 & 10,000 | N.E. | N.E. | - | - | - | - |
| Positive controls: | | | | | | |
| 4-NQO | 0.5 | - | 0.8 | - | 0.1 | - |
| Endoxan | - | 2.2 | - | 2.3 | - | 1.3 |

N.E. = Not Evaluable

Mitotic Gene Conversion

(Mean number of tryptophane revertants/plate)

| Concentration of Isopropoxyphenol in ug/ml: | I w/o S-9 | I with S-9 | II w/o S-9 | II with S-9 | III w/o S-9 | III with S-9 |
|---|---|---|---|---|---|---|
| 0 | 25 | 25 | 35 | 28 | 13 | 30 |
| 185.9 | - | - | - | - | 14 | - |
| 187.5 | - | - | 32 | 29 | - | - |
| 260.3 | - | - | - | - | 15 | - |
| 364.4 | - | - | - | - | 11 | - |
| 375 | - | - | 36 | 29 | - | - |
| 510.2 | - | - | - | - | 14 | 18 |
| 625 | 20 | 26 | - | - | - | - |
| 714.3 | - | - | - | - | 10 | 14 |
| 750 | - | - | 31 | 27 | - | - |
| 1,000 | - | - | - | - | 8 | 18 |
| 1,250 | 24 | 28 | - | - | - | - |
| 1,400 | - | - | - | - | - | 4 |
| 1,500 | - | - | 21 | 21 | - | - |
| 1,960 | - | - | - | - | - | 4 |
| 2,500 | 17 | 14 | - | - | - | - |
| 2,744 | - | - | - | - | - | 1 |
| 3,000 | - | - | 9 | N.E. | - | - |
| 5,000 | N.E. | 6 | - | - | - | - |
| 10,000 | N.E. | 0 | - | - | - | - |
| Positive controls: | | | | | | |
| 4-NQO | 45 | - | 278 | - | 54 | - |
| Endoxan | - | contaminated | - | 65 | - | 84 |

Discussion:

While this was not an outstanding study (the 4-NQO was not an appropriate positive control for mitotic crossing-over), we can accept it as showing that the test material:

1. At exposure levels of up to 3000 ug/ml, without S-9 mix, does not increase the incidence of mitotic cross-overs.

2. At exposure levels of up to 1000 ug/ml, with S-9 mix, does not increase the incidence of mitotic cross-overs.

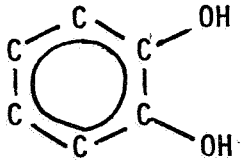
3. At exposure levels of up to 3000 ug/ml, with or without S-9 mix, does not increase the incidence of gene conversion.

Data Evaluation Report (III)

004231

Compound:

Brenzcatechin (1,2-dihydroxybenzene; a metabolite of Propoxur)



Citation:

Herbold, B. Brenzcatechin Salmonella/microsome test to evaluate for potential point mutation. Report no. 12322, control no. 88507, 12-20-83. Study conducted at the Bayer AG Institute of Technology. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus 12/28/84

Byron T. Backus
Toxicologist
Toxicology Branch

Approved by:

1/14/85

Robert Zendzian, Ph.D.
Acting Section Head, Section III
Toxicology Branch

Core Classification: Acceptable

Conclusions:

1. There was no indication of any mutagenic activity as a result of exposure to the test material at concentrations of up to 2500 ug/plate without S-9 mix, or at concentrations of up to 10000 ug/plate with S-9 mix.

2. Above 2500 ug/plate (1250 ug/plate for TA 1535) test material without S-9 mix there was bacteriotoxicity. Depending on the Salmonella strain, toxicity with S-9 mix occurred at levels ranging from 2500-12500 ug/plate of test material.

Materials:

Brenzcatechin, batch VK 32-800.

Positive controls: Endoxan (a bifunctionally alkylating agent after activation); trypaflavine (batch 4899905; a frameshift promutagen); 2-amino-anthracene (batch 10630; a reversing promutagen).

Salmonella typhimurium LT2; TA 1535, TA 1537, TA 100 and TA 98.

S-9 mix, produced from the livers of at least 6 adult male Sprague-Dawley rats, given a single IP injection of aroclor 1254 (dose of 500 mg/kg body weight) dissolved in peanut oil five days before preparation.

20

004231

Procedure:

Demineralized water was the solvent for Brenzcatechin and Endoxan; DMSO was the solvent for trypaflavine and 2-amino-anthracene. Positive controls for the Salmonella typhimurium strains TA 1535 and 100 were Endoxan and 2-amino-anthracene (2-AA). For TA 1537 and TA 98 positive controls were trypaflavine and 2-AA.

Four agar plates per strain were used per substance and dose. In all groups, bacterial counts were determined from 2 or more plates. Testing at all levels of the test material was both with and without S-9 mix.

There were two series of tests. In the first, mutagenicity of brenzcatechin was evaluated at 0 (negative control), 20, 100, 500, 2500 and 12500 ug/plate. In the second, it was evaluated at 0, 625, 1250, 2500, 5000 and 10000 ug/plate.

Results:

From Tables 1 - 8, p. 14-21.

Mean number of revertant colonies formed with strains (first series)

| Compound | Dose level (ug/plate) | TA 1535 -S9 | TA 1535 +S9 | TA 100 -S9 | TA 100 +S9 | TA 1537 -S9 | TA 1537 +S9 | TA 98 -S9 | TA 98 +S9 |
|---|---|---|---|---|---|---|---|---|---|
| Brenzcatechin | 0 | 7 | 8 | 52 | 90 | 3 | 3 | 16 | 23 |
| | 20 | 5 | 9 | 45 | 86 | 3 | 6 | 15 | 17 |
| | 100 | 4 | 8 | 40 | 86 | 2 | 3 | 15 | 24 |
| | 500 | 3 | 12 | 55 | 101 | 5 | 4 | 19 | 24 |
| | 2500 | 2 | 5 | 51 | 90 | 5 | 7 | 18 | 22 |
| | 12500 | ** | 4 | 77 | 10** | 0** | 5 | 0** | 15 |
| Endoxan | 145 | 6 | 108 | 48 | 267 | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 83** | 398 | 82 | 644 |
| 2-amino-anthracene | 3 | 5 | 56 | 77 | 310 | 5 | 26 | 40 | 262 |

**Bacteriotoxic effect.

Mean number of revertant colonies formed with strains (second series)

| Compound | Dose level (ug/plate) | TA 1535 -S9 | TA 1535 +S9 | TA 100 -S9 | TA 100 +S9 | TA 1537 -S9 | TA 1537 +S9 | TA 98 -S9 | TA 98 +S9 |
|---|---|---|---|---|---|---|---|---|---|
| Brenzcatechin | 0 | 8 | 20 | 57 | 84 | 6 | 14 | 17 | 26 |
| | 625 | 5 | 8 | 63 | 96 | 5 | 10 | 23 | 28 |
| | 1250 | 4 | 7 | 64 | 94 | 7 | 12 | 16 | 27 |
| | 2500 | 3** | 15 | 63 | 78 | 6 | 8 | 5 | 31 |
| | 5000 | ** | 7 | ** | 77 | ** | 7 | ** | 22 |
| | 10000 | 0** | 5 | 0** | 31 | 0(**) | 9 | 0(**) | 21 |
| Endoxan | 145 | 6 | 253 | 55 | 314 | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 51 | 1026 | 22 | 1336 |
| 2-amino-anthracene | 3 | 7 | 241 | 79 | 556 | 11 | 92 | 20 | 457 |

** Bacteriotoxic effect.

(**) A bacteriotoxic effect presumably occurred but was not reported.

004231

Discussion:

There were no indications of mutagenic effects (including base pair substitutions or frameshifts) in any of the four strains of Salmonella typhimurium tested. The test material was bacteriotoxic at concentrations above 2500 ug/plate without the S-9 mix. Bacteriotoxicity tended to occur at higher concentrations (5000 to above 10000 ug/plate) of test material when S-9 mix was used.

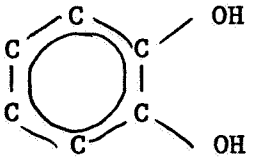
The study is acceptable.

Data Evaluation Report (IV)

004231

Compound:

Brenzcatechin (1,2-dihydroxybenzene; a metabolite of Propoxur)




Citation:

Herbold, B. Pol test on E. coli to evaluate for potential DNA damage. Report no. 12497, control no. 88508, 2-29-84. Study conducted at the Bayer AG Institute of Technology. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Byron T. Backus 01/08/85

Approved by:

1/17/85

Robert Zendzian, Ph.D.
Acting Section Head, Section III
Toxicology Branch

Core Classification: Acceptable

Product Classification: N/A

Conclusions:

1. The study is acceptable in demonstrating that exposure to the test material, at concentrations of up to 6075 ug/plate, produces no damage to E. coli DNA either with or without S-9 mix.

Materials:

Brenzcatechin, batch VK 32-800

Chloramphenicol, batch 2124391080 (used as negative control).

Methyl methane sulphonate (=MMS), batch 1171879.

S-9 mix, produced from the livers of at least 6 adult male Sprague-Dawley rats, given a single IP injection of aroclor 1254 (dose of 500 mg/kg body weight) dissolved in peanut oil five days before preparation.

E. coli, strains (K 12)p 3478 (with a repair deficiency) and W 3110 (pol A+).

23

Procedure:

The appropriate amounts of test material were put on round filter papers, which were then placed on agar plates which had been previously inoculated with bacteria. Four plates were used per substance, dose and strain, both with and without S-9 mix.

In the first part of the study brenzcatechin was tested at 0, 625, 1250, 2500, and 5000 ug. At 10000 ug the test material was not soluble and it was therefore not tested at this level.

In the second and third parts of the study brenzcatechin was tested at 0, 1200, 1800, 2700, 4050 and 6075 ug/plate. In the fourth part of the study brenzcatechin was tested at 0, 800, 1200, 1800, 2700 and 4050 ug/plate.

Chloramphenicol at 30 ug/plate was negative control and MMS at 10 ul per plate was positive control in each part of the study.

After incubation at 37° C for 24 hrs the diameters of the zone of inhibition were measured in mm.

Results:
Only the means of the plates are reported:
(From tables 1, 3, 5, 7):

Zones of inhibition (in mm)
Without S-9 Mix

| | run I | | | run II | | | run III | | | run IV | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Material | rep. | rep. | Diff. | rep. | rep. | Diff. | rep. | rep. | Diff. | rep. | rep. | Diff. |
| (ug/plate) | - | + | | - | + | | - | + | | - | + | |
| Solvent control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brenzcatechin | | | | | | | | | | | | |
| 625 | 0 | 0 | 0 | - | - | - | - | - | - | - | - | - |
| 800 | - | - | - | - | - | - | - | - | - | 0 | 0 | 0 |
| 1200 | - | - | - | 17.2 | 15.9 | +1.3 | 12.2 | 10.9 | +1.3 | 16.0 | 14.9 | +1.1 |
| 1250 | 15.0 | 14.0 | +1.0 | - | - | - | - | - | - | - | - | - |
| 1800 | - | - | - | 18.6 | 20.1 | -1.5 | 15.9 | 15.9 | 0 | 19.1 | 20.3 | -1.2 |
| 2500 | 21.0 | 18.2 | +2.8 | - | - | - | - | - | - | | | |
| 2700 | - | - | - | 21.6 | 23.0 | -1.4 | 19.4 | 19.8 | -0.4 | 22.4 | 23.6 | -1.2 |
| 4050 | - | - | - | 21.3 | 24.9 | -3.6 | 22.4 | 24.0 | -1.6 | 25.5 | 25.3 | +0.2 |
| 5000 | 24.8 | 22.8 | +2.0 | - | - | - | - | - | - | - | - | - |
| 6075 | - | - | - | 27.7 | 28.1 | -0.4 | 24.7 | 26.5 | -1.8 | - | - | - |
| 10000 | Not soluble | | | - | - | - | - | - | - | - | - | - |
| CAP 30 | 24.2 | 29.2 | -5.0 | 21.5 | 30.8 | -9.3 | 23.1 | 27.4 | -4.3 | 21.1 | 27.9 | -6.8 |
| MMS 10 ul | 62.9 | 47.1 | +15.8 | 54.5 | 43.6 | +10.9 | 51.1 | 32.6 | +18.5 | 62.4 | 47.4 | +15.0 |

(From tables 2, 4, 6, 8):

| | With S-9 Mix | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | run I | | | run II | | | run III | | | run IV | | |
| Material | rep. | rep. | Diff. | rep. | rep. | Diff. | rep. | rep. | Diff. | rep. | rep. | Diff. |
| (ug/plate) | - | + | | - | + | | - | + | | - | + | |
| Solvent control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brenzcatechin | | | | | | | | | | | | |
| 625 | 9.0 | 9.3 | -0.3 | - | - | - | - | - | - | - | - | - |
| 800 | - | - | - | - | - | - | - | - | - | 0 | 0 | 0 |
| 1200 | - | - | - | 15.8 | 15.6 | +0.2 | 12.0 | 11.5 | +0.5 | 15.0 | 16.1 | -1.1 |
| 1250 | 12.9 | 14.2 | -1.3 | - | - | - | - | - | - | - | - | - |
| 1800 | - | - | - | 16.8 | 19.4 | -2.6 | 15.5 | 15.9 | -0.4 | 19.8 | 20.0 | -0.2 |
| 2500 | 16.0 | 19.6 | -3.6 | - | - | - | - | - | - | - | - | - |
| 2700 | - | - | - | 18.9 | 21.9 | -3.0 | 17.8 | 21.2 | -3.4 | 23.1 | 23.1 | 0 |
| 4050 | - | - | - | 23.2 | 24.6 | -1.4 | 20.2 | 22.9 | -2.7 | 24.2 | 27.1 | -2.9 |
| 5000 | 26.5 | 26.3 | +0.2 | - | - | - | - | - | - | - | - | - |
| 6075 | - | - | - | 26.5 | 26.7 | -0.2 | 26.4 | 28.6 | -2.2 | - | - | - |
| 10000 | Not soluble | | | - | - | - | - | - | - | - | - | - |
| CAP 30 | 26.9 | 26.7 | +0.2 | 21.1 | 28.3 | -7.2 | 20.4 | 25.2 | -4.8 | 22.3 | 28.9 | -6.6 |
| MMS 10 ul | 59.8 | 43.0 | +16.8 | 54.8 | 42.2 | +12.6 | 52.2 | 40.7 | +11.5 | 61.3 | 42.0 | +19.3 |

Discussion:

The test material inhibits both the pol A+ and the repair-deficient E. coli equally, with and without S-9 activation. There is no evidence then that the test substance damages DNA in E. coli.

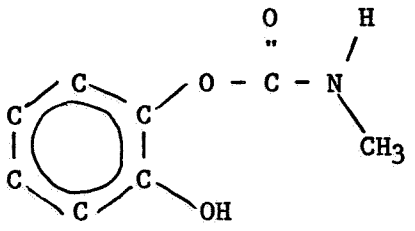
Although only the means of the plates per dose were reported, the study is acceptable.

004231

Data Evaluation Report (V)

Compound:

2-Hydroxyphenyl-methylcarbamate (THS 2490, M3 metabolite of Propoxur)



Citation:

Herbold, B. THS 2490 Salmonella/microsome test to evaluate for point mutation. Report no. 12529, control no. 88509, 3-6-84. Study conducted at the Bayer AG Institute of Toxicology. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus Byron T. Backus 01/08/85
Toxicologist
Toxicology Branch

Approved by:

Robert Zendzian, Ph.D. 1/14/85
Acting Section Head, Section III
Toxicology Branch

Core Classification: Acceptable

Conclusions:

1. The study is acceptable. There are no indications of any mutagenic effects caused by exposure to concentrations of up to 5000 ug/plate of test material, either with or without S-9 activation, in any of the Salmonella typhimurium strains normally used in this type of study.

Materials:

THS 2490, lot no. 3111.

Positive controls: Endoxan (a bifunctionally alkylating agent after activation), trypaflavine (batch 4899905; a frameshift promutagen); 2-amino-anthracene (batch 10630; a reversing promutagen).

Histidine-deficient mutants of Salmonella typhimurium LT2; TA 1535, TA 1537, TA 1538, TA 100, TA 98.

S-9 mix, produced from the livers of at least 6 adult male Sprague-Dawley rats, given a single injection of aroclor 1254 (dose of 500 mg/kg) dissolved in peanut oil five days before preparation.

Procedure:

Four agar plates per strain were used per substance and dose, and with and without S-9 mix. Doses tested were 0, 312.5, 625, 1250, 2500 and 5000 ug/plate of THS 2490, 145 ug/plate Endoxan as positive control (for TA 1535 only), 290 ug/plate Endoxan as positive control (TA 100 only), 50 ug/plate trypaflavine (TA 1537, TA 1538, and TA 98), and 3 ug/plate 2-amino-anthracene as positive control.

Buffer titrisol (pH 4.0) was used as solvent for the THS 2490 and Endoxan; and DMSO was used for trypaflavine and 2-amino-anthracene.

The study was run twice.

Results (run I): Mean number of revertant colonies formed with strains

| Compound: | Dose level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | | TA 1538 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 7 | 13 | 65 | 86 | 3 | 6 | 8 | 18 | 3 | 9 |
| THS 2490 | 312.5 | 9 | 13 | 62 | 79 | 3 | 5 | 12 | 16 | 6 | 10 |
| | 625 | 7 | 14 | 64 | 89 | 6 | 8 | 9 | 15 | 6 | 10 |
| | 1250 | 7 | 9 | 60 | 97 | 8* | 6 | 12 | 20 | 4 | 7 |
| | 2500 | 10 | 13 | 56 | 110 | 4 | 6 | 9 | 19 | 4 | 6 |
| | 5000 | 7 | 15 | 49 | 87 | 3 | 4 | 7 | 14 | 6 | 7 |
| Endoxan | 145 | 13 | 195* | - | - | - | - | - | - | - | - |
| | 290 | - | - | 69 | 387* | - | - | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 27* | 767* | 29* | 1802* | 14* | 1420* |
| 2-AA | 3 | 12 | 340* | 136* | 1387* | 12* | 99* | 21* | 709* | 9* | 726* |

| Results (run II): | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | - | 5 | 15 | 61 | 93 | 4 | 6 | 15 | 18 | 7 | 15 |
| THS 2490 | 312.5 | 6 | 12 | 65 | 88 | 6 | 6 | 13 | 16 | 8 | 8 |
| | 625 | 5 | 13 | 63 | 109 | 5 | 4 | 15 | 17 | 8 | 7 |
| | 1250 | 6 | 11 | 54 | 102 | 4 | 5 | 13 | 11 | 4 | 5 |
| | 2500 | 4 | 15 | 59 | 91 | 3 | 6 | 8 | 21 | 5 | 23 |
| | 5000 | 4 | 13 | 57 | 88 | 5 | 4 | 7 | 11 | 6 | 11 |
| Endoxan | 145 | 8 | 295* | - | - | - | - | - | - | - | - |
| | 290 | - | - | 63 | 419* | - | - | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 20* | 569* | 28 | 1777* | 12 | 1232* |
| 2-AA | 3 | 15* | 285* | 117 | 1156 | 11* | 56* | 29 | 515* | 9 | 561* |

* = double the negative control; possible mutagenic effect.

An increase in mutant counts to double that of the negative control was noted with THS 2490 on only one occasion, involving TA 1537 at 1250 ug /plate on the first run. There was no indication of a mutagenic effect at higher dose levels (2500 & 5000 ug/plate), so the result is reported (and can be considered as) a random occurrence.

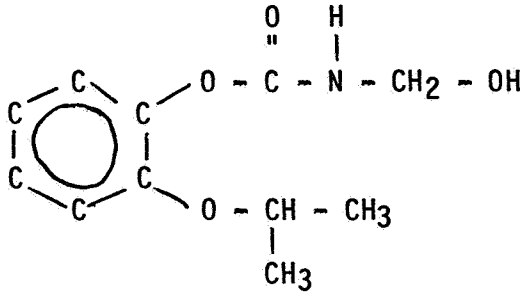
Discussion:

The study is acceptable as demonstrating that the test material shows no mutagenic activity in various strains of S. typhimurium at up to 5000 ug/plate with and without S-9 mix.

004231

Compound:

2-Isopropoxyphenyl-hydroxymethylcarbamate (THS 1241b, M5 metabolite of Propoxur)



Citation:

Herbold, B. THS 1241b Salmonella/microsome test to evaluate for potential point mutation. Report No. 12795; control no. 88510, 9-7-84. Study conducted at Bayer AG Institute of Toxicology. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus Byron T. Backus
Toxicologist 01/03/85
Toxicology Branch

Approved by:

Robert Zendzian, Ph.D.
Acting Section Head, Section III
Toxicology Branch

Study Classification: Supplementary

Conclusions:

1. The study is supplementary. There appears to be weak mutagenicity without activation in S. typhimurium strain TA 1535. If mutagenicity exists it may be due to an impurity; two batches of test material were used and batch 3280 appeared to be somewhat more mutagenic than batch 17101983. However, only strain TA 1535 (with a base pair substitution causing histidine deficiency) was tested using batch 3280.

2. There was no evidence of any frameshift mutation effects as a result of exposure at up to 5000 ug/plate test material (batch 17101983).

Materials:

THS 1241b, batches 17101983 and 3280.

Positive controls, Endoxan (a bifunctionally alkylating agent after activation), trypaflavine (batch 4899905; a frameshift promutagen); and 2-amino-anthracene (batch 10630, a reversing promutagen).

Histidine-deficient mutants of Salmonella typhimurium LT2: TA 1535,

TA 1537, TA 1536, TA 100, TA 98.

004231

S-9 mix, produced from the livers of at least 6 adult male Sprague-Dawley rats, given a single injection of aroclor 1254 (dose of 500 mg/kg) dissolved in peanut oil five days before preparation.

Procedure:

Four agar plates per substance and dose, both with and without S-9 mix. Only the TA 1535 strain was tested at some concentrations of the test material. Concentrations tested and positive controls are reported in results (below).

Results (dosage series I): Mean number of revertant colonies formed/plate/strain:
Date of test: December 5, 1983

| Compound: | Dose level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | |
|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 6 | 7 | 61 | 62 | 4 | 6 | 11 | 12 |
| THS 1241b | 8 | 8 | 8 | 46 | 54 | 7 | 10 | 15 | 14 |
| | 40 | 9 | 9 | 58 | 42 | 9* | 13 | 14 | 16 |
| | 200 | 12* | 14* | 60 | 53 | 5 | 10 | 14 | 14 |
| Endoxan | 145 | 7 | 100* | - | - | - | - | - | - |
| | 290 | - | - | 58 | 196* | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 48* | 508* | 54* | 1718* |
| 2-Amino-anthracene | 3 | 8 | 139* | 91 | 700* | 10 | 72* | 31* | 385* |

*twice or more the mean number (before rounding off) of revertants/plate observed in the negative control.

(Dosage series II): Mean number of revertant colonies formed/plate/strain:
Date of test: Dec. 12, 1983 (except for second TA 1535; Dec. 15, 1983)

| Compound: | Dose level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | | TA 1535 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 4 | 8 | 66 | 58 | 4 | 6 | 10 | 11 | 9 | 7 |
| THS 1241b | 50 | 5 | 4 | 47 | 45 | 4 | 6 | 7 | 19 | 7 | 10 |
| | 100 | 5 | 5 | 50 | 47 | 2 | 7 | 9 | 14 | 6 | 11 |
| | 200 | 5 | 3 | 57 | 73 | 4 | 4 | 7 | 10 | 4 | 12 |
| | 400 | 9* | 5 | 46 | 52 | 3 | 4 | 6 | 11 | 7 | 14 |
| | 800 | 8 | 8 | 71 | 41 | 4 | 4 | 10 | 11 | 10 | 17 |
| Endoxan | 145 | 5 | 70* | - | - | - | - | - | - | 6 | 160* |
| | 290 | - | - | 60 | 136* | - | - | - | - | - | - |
| Trypaflavine | 50 | - | - | - | | 38* | 146* | 18 | 1285* | - | - |
| 2-Amino-anthracene | 3 | 6 | 143* | 68 | | 9* | 43* | 15 | 492* | 7 | 245* |

*twice or more the mean number (before rounding off) of revertants/plate observed for the negative control.

004231

(Dosage series III): Mean number of revertant colonies formed/plate/strain

Date of test: January 30, 1984

| Compound: | Dose level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | | TA 1538 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 4 | 13 | 79 | 96 | 5 | 6 | 13 | 16 | 4 | 8 |
| THS 1241b | 312.5 | 11* | 12 | 71 | 92 | 6 | 5 | 9 | 18 | 4 | 6 |
| | 625 | 7 | 15 | 66 | 108 | 5 | 9 | 10 | 10 | 5 | 5 |
| | 1250 | 11* | 19 | 83 | 113 | 4 | 7 | 8 | 8 | 4 | 5 |
| | 2500 | 14* | 19 | 94 | 99 | 4 | 4 | 6 | 7 | 5 | 5 |
| | 5000 | 16* | 21 | 95 | 92 | 5 | 4 | 7 | 8 | 4 | 2 |
| Endoxan | 145 | 7 | 226* | - | - | - | - | - | - | - | - |
| | 290 | - | - | 78 | 357* | - | - | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 15* | 205* | 23 | 1741* | 13* | 1431* |
| 2-Amino-anthracene | 3 | 15 | 243 | 154 | 1164* | 20* | 98* | 33* | 655* | 9* | 649* |

*twice or more the mean number (before rounding off) of revertants/plate observed for the negative control.

(Dosage series IV): Mean number of revertant colonies formed/plate/strain

Date of test: February 9, 1984

| Compound: | Dose level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | |
|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 5 | 15 | 61 | 93 | 4 | 6 | 15 | 18 |
| THS 1241b | 312.5 | 4 | 13 | 53 | 92 | 4 | 6 | 10 | 16 |
| | 625 | 4 | 14 | 62 | 92 | 4 | 5 | 10 | 17 |
| | 1250 | 9 | 17 | 68 | 86 | 3 | 5 | 10 | 21 |
| | 2500 | 12* | 24 | 67 | 89 | 4 | 6 | 13 | 16 |
| | 5000 | 14* | 35* | 67 | 76 | 6 | 5 | 11 | 16 |
| Endoxan | 145 | 8 | 295* | - | - | - | - | - | - |
| | 290 | - | - | 63 | 419 | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 20* | 569* | 28 | 1777* |
| 2-Amino-anthracene | 3 | 15* | 285* | 117 | 1156 | 11* | 56* | 29 | 515* |

*twice or more the mean number (before rounding off) of revertants/plate observed for the negative controls.

(Dosage series V): Mean number of revertant colonies formed/plate
Date of test (i): February 17, 1984
Date of test (ii): February 24, 1984.
Date of test (iii, iv, v): January 6, 1984

| Compound | Dose level (ug/plate) | (i) TA 1535 | | (ii) TA 1535 | | (iii) TA 1535 | | (iv) TA 1535 | | (v) TA 1535 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 7 | 11 | 9 | 14 | 14 | 10 | 19 | 8 | 21 | 9 |
| THS 1241b** | 1200 | 11 | 21 | - | - | - | - | - | - | - | - |
| | 1800 | 14* | 15 | 10 | 17 | 46* | 21* | 52* | 17* | 61* | 20* |
| | 2700 | 12 | 23* | 8 | 17 | 49* | 23* | 53* | 13 | 68* | 26* |
| | 4050 | 16* | 31* | 13 | 17 | 24 | 17 | 51* | 14 | 62* | 24* |
| | 6075 | 13 | 32* | 16 | 26 | 25 | 27* | 43* | 19* | 48* | 19* |
| | 7290 | - | - | 17 | 19 | 22 | 34* | 43* | 14 | 39 | 20* |
| | 8748 | - | - | 12 | 27 | 29* | 28* | 22 | 11 | 30 | 19* |
| Endoxan | 145 | 8 | 295* | 10 | 350* | 17 | 146* | 18 | 95* | 23 | 127* |
| 2-Amino-anthracene | 3 | 9 | 333* | 8 | 290* | 19 | 219* | 19 | 219* | 19 | 219* |

*twice or more the number (before rounding off) of revertants observed/plate in the negative controls.

THS 1241b batch 17101983 was used for (i) and (ii)
THS 1241b batch 3280 was used for (iii), (iv) and (v).

Discussion:

It seems obvious that the incidence of mutations in TA 1535 increased on exposure to the test material, often without, but sometimes with, S-9 mix. The increase is particularly apparent for the plates done January 6, 1984 when THS 1241b batch 3280 was used. Although a dose-relationship is not readily apparent, it may be that at these levels of test material there was essentially "saturation." The drop in mutations at the higher concentrations (7290 and 8748 ug/plate) may simply reflect "cytotoxicity" or inhibition.

Since TA 100 and TA 1535 contain the same base-substitution, TA 100 might be expected to also show an increase in mutation rate on exposure to the test material. A slight increase of about 20% in mutation rates on exposure to concentrations of 2500 and 5000 ug/plate (highest levels tested with TA 100) of the test material did occur in the plates of January 30, 1984. However, this is far below the doubling of the negative control mean plate count used as indicating a possible mutagenic effect in this study.

No testing was done exposing S. typhimurium strain TA 100 (or any other strain, except TA 1535) to THS 1241b batch 3280.

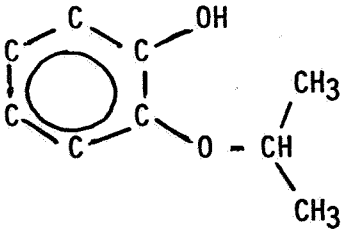
Although no evidence for frameshift mutations was observed as a result of exposure to up to 5000 ug/plate of test material (batch 17101983 only) the test strains were exposed to 1250, 2500 and 5000 ug/plate only on one occasion (Jan. 30, 1984), so this part of the study was not done in duplicate.

Data Evaluation Report (VII)

004231

Compound:

Isopropoxyphenol (M2 metabolite of propoxur)



Citation:

Herbold, B. Isopropoxyphenol Salmonella/microsome test to evaluate for point mutation. Report no. 12321; control no. 88581; 12-20-83. Study conducted at the Bayer AG Institute of Toxicology. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus 12/28/84
Toxicologist
Toxicology Branch

Approved by:

1/14/85
Robert Zendzian, Ph.D.
Acting Section Head, Section III
Toxicology Branch

Study Classification: Acceptable

Conclusions:

1. The test material causes no mutagenic effects, with or without S-9 mix, in Salmonella typhimurium strains TA 1535, TA 100, TA 1537 and TA 98, at concentrations of up to 2500 ug/plate.

2. At 5000 ug/plate there was no indication of mutagenic activity in the test organisms; however, this level was tested only once.

3. At 10000 ug/plate and above the test material was bacteriotoxic.

Materials:

Isopropoxyphenol, batch VK 32-806.

Positive controls: Endoxan (batch 092416, a bifunctionally alkylating agent after activation); trypaflavine (batch 4899905, a frameshift promutagen); and 2-amino-anthracene (batch 10630; a reversing promutagen).

Histidine-deficient mutants of Salmonella typhimurium LT2: TA 1535, TA 1537, TA 100, and TA 98.

S-9 mix, produced from the livers of at least 6 adult male Sprague-Dawley rats, given a single injection of aroclor 1254 (at 500 mg/kg) dissolved in peanut oil five days before preparation.

Procedure:

Four agar plates were prepared, both with and without S-9 mix, per dose and substance. Levels tested and positive controls are given in results (below).

Results (series I): Mean number of revertant colonies formed/plate/strain

Date of test: October 6, 1983

| Compound: | Dose level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | |
|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 7 | 8 | 52 | 90 | 3 | 3 | 16 | 23 |
| Isopropoxy-phenol | 20 | 5 | 12 | 47 | 81 | 2 | 6 | 17 | 18 |
| | 100 | 8 | 12 | 56 | 88 | 3 | 4 | 14 | 24 |
| | 500 | 8 | 12 | 73 | 83 | 2 | 6 | 20 | 23 |
| | 2500 | 5 | 10 | 30 | 80 | 1 | 5 | 10 | 22 |
| | 12500 | 4† | 6† | 43† | 82† | 3† | 0† | 1† | 0† |
| Endoxan | 145 | 6 | 108* | - | - | - | - | - | - |
| | 290 | - | - | 48 | 267* | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 83* | 398* | 82* | 644* |
| 2-Amino-anthracene | 3 | 5 | 56* | 77 | 310* | 5 | 26* | 40* | 262* |

*twice or more the mean number (before rounding off) of revertants/plate observed in the negative control.
†bacteriotoxic effect at this concentration of test material.

Results (series II): Mean number of revertant colonies formed/plate/strain

Date of test: October 20, 1983

| Compound | Dose Level (ug/plate) | TA 1535 | | TA 100 | | TA 1537 | | TA 98 | |
|---|---|---|---|---|---|---|---|---|---|
| | | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Vehicle | - | 8 | 20 | 57 | 84 | 6 | 14 | 17 | 26 |
| Isopropoxy-phenol | 625 | 6 | 19 | 41 | 82 | 5 | 12 | 17 | 31 |
| | 1250 | 5 | 9 | 56 | 79 | 7 | 8 | 15 | 31 |
| | 2500 | 7 | 8 | 53 | 76 | 4 | 10 | 15 | 35 |
| | 5000 | 4 | 16 | 51 | 66 | 2 | 9 | 8 | 37 |
| | 10000 | 2† | 0† | 29† | 0† | 0† | 0† | 0† | 9† |
| Endoxan | 145 | 6 | 253* | - | - | - | - | - | - |
| | 290 | - | - | 55 | 314* | - | - | - | - |
| Trypaflavine | 50 | - | - | - | - | 51* | 1026* | 22 | 1336* |
| 2-Amino-anthracene | 3 | 7 | 241* | 79 | 556* | 11 | 92* | 20 | 457* |

*twice or more the mean number (before rounding off) of revertants/plate observed for the negative controls.
†bacteriotoxic effect at this concentration of test material.

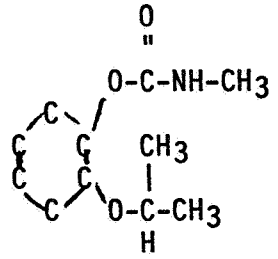
Discussion:

At concentrations greater than 5000 ug/plate of the test material there was a bacteriotoxic effect.

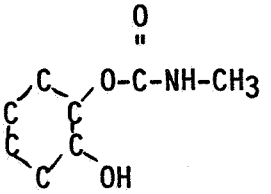
The study is acceptable as showing that the test material causes no mutagenic effects (base pair substitutions, frameshifts) in Salmonella typhimurium normally used in this protocol at up to 2500 ug/plate with and without S-9 activation (although there were no indications of mutagenic effects at 5000 ug/plate, this concentration was tested only once).

Compounds:

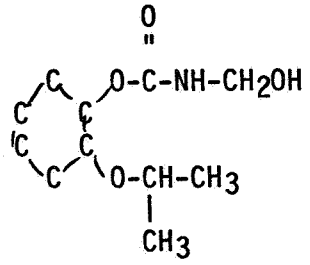
Propoxur (Baygon):



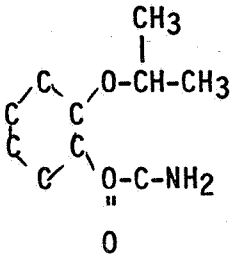
THS 2490:



THS 1241b:



THS 1240:



Study type:

Mutagenicity (DNA metabolism) - rat spleen cells

Citation:

Klein, W., Kocsis, F., Lippl, K., Schwarzinger, B. and Bornatowicz, N. Effect of an active ingredient and three metabolites on the DNA metabolism. Study conducted at the Institut für Biologie, Forschungszentrum Seibersdorf, (Austria); study commissioned by Bayer AG. OEFZS Report no. A050, BL-453/84. Control no. 88582; report dated March 1984. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus 12/28/84
Toxicologist
Toxicology Branch

Approved by:

Robert Zendzian, Ph.D.
Acting Section Head, Section III
Toxicology Branch

Core Classification: Supplementary

Conclusions:

1. In terms of the protocol this seems to be a somewhat unique study. It does not satisfy any Agency requirements. It is interesting that two of the metabolites (THS 2490 and THS 1241b) have a suppressive effect on programmed DNA synthesis in spleen cells, but it is difficult to make

any firm conclusions as to what this means. Perhaps it relates in some way to the higher incidence of splenic degeneration that was observed in rats at the 5000 ppm dietary exposure level in in the 2-year feeding study.

2. This study utilized spleen cells, and single dose exposure. The organs in the 2-year study which showed oncogenic effects were the bladder and uterus.

3. No justification is made as to why only a single exposure level (10 mg/kg) of each of these compounds was administered.

Materials:

BOQ 5 812 315 (identified as the active ingredient), THS 2490, THS 1240 and THS 1241b (the last 3 being metabolites), as received 12th December 1983 from Bayer AG. The positive control was cyclophosphamide, SERVA Co., 17 681.

Male rats (BOR:WISW, SPF), supplied by Winkelmann Versuchstierzucht GmbH, D-4799, Borchen.

Procedure:

Groups of 24 (21 for the positive control group receiving cyclophosphamide) rats received p.o. either 10% ethanol solution (negative controls), 10 mg/kg of BOQ 5 812 315, 10 mg/kg THS 1240, 10 mg/kg THS 2490, 10 mg/kg THS 1241b, or 100 mg/kg cyclophosphamide in physiological NaCl solution.

Twenty-four hours later the rats were sacrificed, spleens were removed, and spleen cell suspensions were prepared. Spleen cell suspensions from different animals were not combined.

Programmed DNA synthesis: 2.5 uCi $^{3}H$-thymidine was added to an aliquot of each of the spleen suspensions from 12 animals/group. After 2 hours incubation at 37° C cell suspensions were treated with iced 6% perchloric acid (PCA). Cell sediment was washed 3 times with 6% PCA, followed by hydrolysis in 6% PCA at 90° C for 30 minutes. The quantity of DNA in the aliquot was determined photometrically, and the radioactivity was determined by liquid scintillation. The ratio of the radioactivity to amount of DNA present was then calculated.

Suppressed programmed DNA synthesis: "Programmed DNA synthesis is largely suppressed by hydroxyurea (by about 90%). The DNA radioactivity which is nevertheless measurable is the result, firstly of the residual DNA synthesis still present, and secondly of continuous DNA repairs."

Aliquots of cell suspensions were pre-incubated for 30 minutes at 37° C with $10^{-2}$ M hydroxyurea. They were then treated as indicated in the procedure for programmed DNA synthesis above, and quantity of DNA present and radioactivity of the sample were determined.

Unprogrammed DNA synthesis (DNA repair): Cell suspensions were preincubated with $10^{-2}$ M hydroxyurea for 30 minutes at 37° C. The suspensions were then subjected to UV(254 nm) light at 20 J/m$^2$. The cell suspensions were then

treated with $^{3}H$-thymidine for 120 minutes, and the procedure followed that given above under Programmed DNA synthesis.

Nucleoid sedimentation: "DNA of cells lysed in the presence of non-ionic detergents and high salt concentrations not only retains the secondary structure of the double helix, but also the tertiary structure of the super-helix... Any loss of supercoils, say by DNA inclusions, strand breaks or intercalation...results in a clearly changed sedimentation pattern...in the saccharose gradients."

After lysis in a salt + non-ionic detergent (Triton X-100) mixture, cells were subjected to ultra-centrifugation in salt-saccharose solutions. Sedimentation profiles of the gradients were established using a photo-metric analysis at 254 nm. Both unradiated and radiated (cobalt 60) DNA from the spleen cells were analyzed.

Substance-DNA binding tests: "Isolated cleaned DNA is incubated with a test compound. The binding of the test compound to the DNA can be determined after incubation by appropriate methods (radioactivity measurement, HPLC, UV spectroscopy etc.)."

According to the protocol (p. 13): DNA was extracted from "the weighed mouse liver" ("mouse" is probably a misprint for "rat") of a control group which had received only ethanol. Five mg of each of the test compounds were dissolved in a mixture of 96% ethanol and 0.1 SSC (standard salt-citrate solution) to which was added 1067 ug of isolated cleaned rat DNA in 0.1 SSC solution. The mixture was incubated at 37° C for 24 hrs. After a fairly complex analytical procedure involving determination of amounts of DNA present and preparation of standards, the DNA-substance binding was checked with an HPLC.

Statistics:

Variations between the six groups examined were checked for statistical relevance with a 2-sided "T" test.

Results:
Programmed DNA Synthesis:
From p. 16:

| Group | Number of Animals | Mean (negative control = 100) | S.D. |
|---|---|---|---|
| Negative Control | 12 | 100 | 20 |
| Baygon | 12 | 92 | 17 |
| THS 1240* | 10 | 91 | 21 |
| THS 1240 (all animals) | 12 | 86 | 23 |
| THS 2490 | 12 | 83†† | 14 |
| THS 1241b** | 11 | 85† | 18 |
| THS 1241b (all animals) | 12 | 93 | 32 |
| Cyclophosphamide | 12 | 28†† | 7 |

*Data from two animals not included in calculation.
**Data from one animal not included in calculation.
†p = 0.07
††p < 0.05

Data from certain animals were not included in calculations. Rationale (p. 18) is: "...on the strength of many years of experience in these tests the ratios of the various test parameters appear extremely improbable." 004231

Suppressed Programmed DNA Synthesis:
From p. 17:

| Group | Number of Animals | Mean (negative control = 100) | S.D. |
|---|---|---|---|
| Negative Control | 12 | 100 | 10 |
| Baygon (BOQ 5 812 315) | 12 | 96 | 11 |
| THS 1240 | 12 | 95 | 14 |
| THS 2490 | 12 | 99 | 11 |
| THS 1241b | 12 | 99 | 18 |
| Cyclophosphamide | 12 | 64†† | 13 |

††p < 0.05 by t-test

Unprogrammed DNA Synthesis after UV Radiation:
From p. 20:

| Group | Number of Animals | Mean (negative control = 100) | S.D. |
|---|---|---|---|
| Negative Control | 12 | 100 | 5 |
| Baygon (BOQ 5 812 315) | 12 | 99 | 11 |
| THS 1240 | 12 | 93 | 15 |
| THS 2490 | 12 | 101 | 12 |
| THS 1241b | 12 | 101 | 12 |
| Cyclophosphamide | 12 | 60†† | 16 |

††p < 0.05 by t-test

Nucleoid Sedimentation:
From p. 22:

| Group | Number of Animals | Mean (negative control = 100) | S.D. |
|---|---|---|---|
| Negative Control | 12 | 100 | 4 |
| Baygon (BOQ 5 812 315) | 12 | 99 | 7 |
| THS 1240 | 12 | 97 | 8 |
| THS 2490 | 12 | 98 | 8 |
| THS 1241b | 12 | 97 | 7 |
| Cyclophosphamide | 12 | 75†† | 10 |

††p < 0.05 by t-test

Sedimentation of the nucleoids immediately after exposure of cells to 1 Gy ionizing radiation from cobalt 60 (p.23): 004231

| Group | Number of Animals | Mean (negative control of non-radiated spleen cells = 100) | S.D. |
|---|---|---|---|
| Negative Control | 12 | 69 | 8 |
| Baygon (BOQ 5 812 315) | 12 | 71 | 8 |
| THS 1240 | 12 | 70 | 7 |
| THS 2490 | 12 | 72 | 6 |
| THS 1241b | 12 | 73 | 6 |
| Cyclophosphamide | 12 | 64 | 10 |

Sedimentation of the nucleoids 2 hours after exposure of cells to 1 Gy ionizing radiation from cobalt 60 (p. 23):

| Group | Number of Animals | Mean (negative control of non-radiated spleen cells = 100) | S.D. |
|---|---|---|---|
| Negative Control | 12 | 98 | 5 |
| Baygon (BOQ 5 812 315) | 12 | 97 | 6 |
| THS 1240 | 12 | 98 | 9 |
| THS 2490 | 12 | 98 | 8 |
| THS 1241b | 12 | 98 | 9 |
| Cyclophosphamide | 12 | 70†† | 9 |

††$p < 0.05$

Bound Quantity of Test Compound in ug compound/ug DNA (from table 13, p. 26):

| | Value |
|---|---|
| Baygon (BOQ 5 812 315) | 0.0173 |
| THS 1240 | 0.0253 |
| THS 1241b | 0.0694 |
| THS 2490b | 0.0108 |

Statement is made (p. 26): "...the figure given for THS 1241b is the result of the reduced difference of added high counts... To classify this as an increased biological relevance of THS 1241b binding...is...not justifiable because of the magnitude."

Discussion:

This study is primarily of academic interest. For a number of reasons it is difficult to relate the findings to those of the 2-year rat feeding study. These include:

1) Only spleen and liver cells were used. The target organs for oncogenicity in the 2-year study were bladder and uterus.

2) Each test material was administered at only one dosage level. No indication could be obtained as to what sort of dose-response (if any) existed with this assay system.

3) No justification was given for the one dosage level (10 mg/kg) that was used for the Baygon and its three metabolites.

4) In each case, there was a single dose admnistered 24 hour before the animals were sacrificed, rather than exposure over a period of time, as in the 2-year study.

It is interesting that an effect (some suppression of programmed DNA synthesis in spleen cells) was observed with two of the metabolites (THS 2490 and THS 1241b). It is even possible that this effect may have had something to do with the high incidence of splenic atrophy that was observed in high-dose (5000 ppm) rats in the 2-year feeding study. However, on the basis of this study alone, this reviewer can come to no further conclusions regarding possible oncogenic risks associated with exposure to the test material and/or its metabolites.

Data Evaluation Report (IX)

004231

Compounds:

2-isopropoxy-phenyl-N-methylcarbamate, BOQ 5812315, Propoxur, Baygon

Study type:

Metabolism

Citation:

Eben, A. and Karl, W. Studies on the biotransformation of Propoxur in the rat. Report no. 12866, dated August 17, 1984. Control no. 88584. Study conducted at the Bayer AG EP-FE Institut für Toxikologie, Wupperta l-Elberfeld. Received at EPA 10-23-84; in Acc. 255177.

Reviewed by:

Byron T. Backus Byron T. Backus 01/14/85
Toxicologist
Toxicology Branch

Approved by:

Robert Zendzian, Ph.D.
Acting Section Head
Toxicology Branch

Core Classification: Supplementary

Conclusions:

1. The study is scientifically sound, and is of considerable interest as it demonstrates that the active ingredient is metabolized by a number of different pathways, and many (but not all) of the metabolites are identified. However, there is very little in the way of quantitative information. Also, the study is primarily concerned with the metabolites present in urine. As a result, the study satisfies no Agency data requirements.

Materials:

Test compound: Propoxur, 98.5-98.8% pure, batch no. 234101303. Reference compounds: 1,2-Dihydroxybenzene (VK 32-800, M1); 2-Isopropoxyphenol (VK 32-806, M2); 2-Hydroxyphenyl-methylcarbamate (THS 2490, M3); 2-Isopropoxyphenyl-carbamic acid (THS 1240, M4); 2-Isopropoxyphenyl-hydroxymethylcarbamate (THS 1241, M5).

Male Wistar rats BOR: WISW (SPF/Cpb) supplied by Winkelmann, Borchen, Kreis Paderborn.

004231

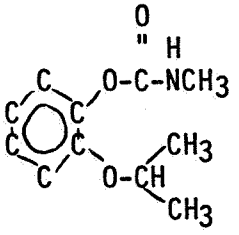
Procedure:

Groups of 10 male rats were maintained on a diet containing either 0 (controls) or 8000 ppm Propoxur. Thirteen weeks after the start of the study treated and control rats were placed singly in metabolism cages and the urine was collected for 24 hours. In order to avoid hydrolysis of some metabolites the pH of the urine was kept at between 4 and 5 by pipetting 0.5 mls 1N HCl into each receiver in the morning and afternoon.

According to the procedure given urine collected from 10 treated rats came to between 80 and 120 mls (interpretation is that the urine samples were pooled). There was a fairly lengthy extraction procedure, with analysis by thin-layer chromatography. Some compounds were identified by comparison with reference compounds, with verification by $^1$H-NMR spectra and mass spectra. Other metabolites, for which no references had been prepared, had to be isolated and purified in quantities of 0.5-3 mg for identification.

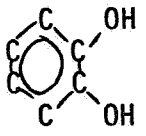
Results:

The test compound (Propoxur, structure given below) was identified in urine:

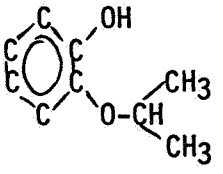


The following metabolites (structure shown below) were also identified in the urine, occurring either free, and/or as glucuronides and sulfates:

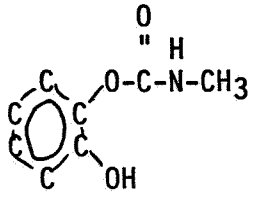
M 1 = 1,2-Dihydroxybenzene, pyrocatechol



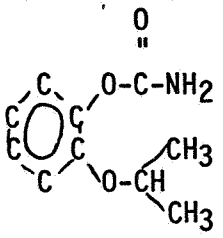
M 2 = 2-isopropoxyphenol:



M 3 = 2-hydroxyphenyl methylcarbamate: (THS 2490)

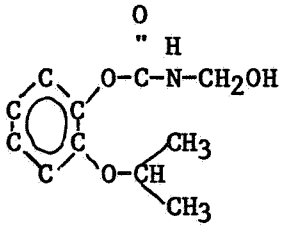


M 4 = 2-isopropoxyphenyl-carbamic acid (THS 1240)



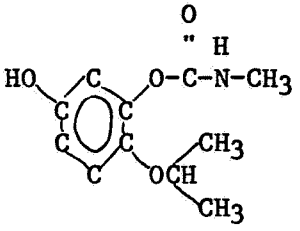
004231

M 5 = 2-isopropoxyphenyl-hydroxymethylcarbamate
(THS 1241)

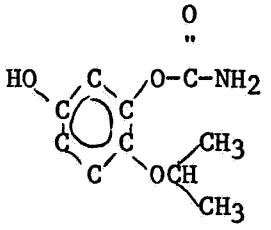


Note: This compound is identified as THS 1241b in some of the other studies.

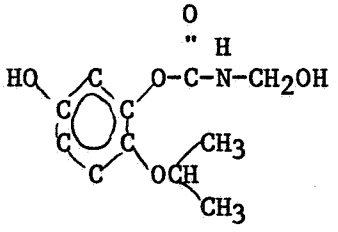
M 6 = 2-isopropoxy-5-hydroxyphenyl-methylcarbamate



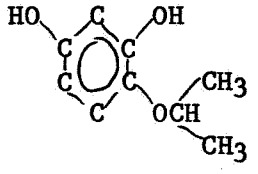
M 6 CII = 2-isopropoxy-5-hydroxyphenyl-carbamic acid



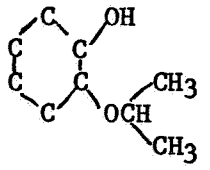
M S 3 = 2-isopropoxy-5-hydroxyphenyl-hydroxymethylcarbamate



M 7 = 1,5-dihydroxy-2-isopropoxy-benzene



M 8 = 1,3-dihydroxy-2-isopropoxy-benzene



The report states (p. 48) that "No data can be given on the concentration of each metabolite."

In addition to the metabolites given above, the report states (p. 46) that several others were detected at very low concentrations, but that it had not been possible to isolate them.

Discussion:

The study is scientifically sound, although somewhat limited in scope. Because radioactive tracers were not used, it was not possible to get quantitative estimates as to how much of the active was metabolized, and how much of each metabolite was produced. The study does not satisfy any Agency data requirements.