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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

To: Henry Jacoby
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From: Roger Gardner
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Thru: Christine F. Chaisson, Ph. D.
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Subject: Review of data on technical grade Terrazole®. EPA Reg. No. 1258-812. Tox. Chem. 428.

Actions Requested

Review of the following studies on technical grade Terrazole®:

81-3 1. An acute inhalation toxicity study in rats
83-3A 2. A teratogenicity study in rats
84-2 3. Two sister chromatid exchange assays
4. A chromosomal aberration assay

Recommendations and Conclusions

1. The LC_{50} for technical grade Terrazole in male and female rats is 7.37 mg/l with 95% confidence limits of 4.40 to 12.35 based on nominal concentration (1). These results place technical grade Terrazole into Toxicity Category III with respect to acute inhalation toxicity.

2. The no-observed-effect levels (NOEL) for maternal and fetal toxicity are 30 mg/kg/day, and the lowest-effect level (LEL) is 75 mg/kg/day (highest dose tested) (2). There were no teratogenic effects caused by Terrazole®.

3. The mutagenicity studies (3 and 4) indicate that concentrations of 20 to 60 ug Terrazole® per ml of growth medium significantly increase the number of sister chromatid exchanges and chromosomal aberrations per Chinese hamster ovary cell.

4. The results described in point 3. above (see appended DATA EVALUATION RECORDS also), and the results of a long-term mouse study reviewed previously (see discussion below) indicate that Terrazole should be recommended for Special Review as a potential oncogen.

Background and Discussion

The four studies reviewed herein were identified as data gaps in the Agency's Registration Standard for Terrazole® which was published in September, 1980. The mutagenicity studies submitted are added to a battery of assays previously reviewed (see the above mentioned Registration Standard and a memorandum dated September 9, 1982. From: R. Gardner. To: H. Jacoby, Registration Division. Subject: Tolerances for Terrazole and its monoacid metabolite in/on corn, meat, milk, eggs, and wheat. PP#0F2317. EPA Acc. Nos. 070605-070613. Reg. Nos. 1258-RNNG (Dwell 95% LC), 1258-RNNU (Dwell 25% L), and 1258-RNNL (Dwell 44% L). Tox. Chem. No. 428). Those assays included reverse mutation studies in Salmonella typhimurium, DNA repair studies in Escherichia coli, and a point mutation assay in Chinese hamster ovary cells (HGPRT locus). All of these tests failed to demonstrate mutagenic activity associated with Terrazole®.

The Registration Standard also described a rabbit teratology study which demonstrated a NOEL for fetal and maternal toxicity of 15 mg/kg/day. The LEL reported from that study was 45 mg/kg/day. Terrazole did not cause terata in that study.

It should also be noted that a statistically significant increase in tumors has been reported in a mouse study (Erker, E. F., L. J. Slaughter, T. F. Holford, and W. L. West. 1981. Terrazole® 18 month oncogenicity bioassay in CD-1 mice: Final Report. Unpublished report no. 3543 prepared by Howard University. Submitted by Olin Corporation. EPA Acc. No. 070606-613.), and the Toxicology Branch has recommended that the study be re-evaluated as part of an assessment of Terrazole's oncogenic potential (Memorandum dated February 4, 1984. From: R. Gardner. To: Henry Jacoby, Registration Division. Subject:Response to the review of an eighteen-month feeding study in mice with Terrazole. Petition No. 0F2317).

The results reported by the mutagenicity studies reviewed herein (see appended DATA EVALUATION RECORDS; references 3 and 4), and the results of a long-term mouse study reviewed previously (see above) indicate that Terrazole should be recommended for special review on the basis of suggested oncogenic potential.

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APPENDIX

The following DATA EVALUATION RECORDS are included in this Appendix

1. Eschbach, J. C., and G. K. Hogan. December 10, 1981. An acute inhalation toxicity study of Terrazole in the rat. Unpublished report no. 3742 prepared by BioDynamics, Inc. Submitted by Olin Corporation. EPA Acc. No. 249073.

2. International Research and Development Corporation. May 27, 1982. Terrazole®: Teratology study in rats. Unpublished report no. 3762. Submitted by Olin Corporation. EPA Acc. No. 249073.

3. Loveday, K. S., and B. A. Donahue. January 26, 1982. In vitro sister chromatid exchange assay on Terrazole®. Unpublished report no. 3745 prepared by Bioassay Systems Corporation. Submitted by Olin Corporation. EPA Acc. No. 249073.

4. Loveday, K. S. June 4, 1982. The effects of Terrazole® on the In vitro induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. Unpublished report no. 3765 prepared by Bioassay Systems Corporation. Submitted by Olin Corporation. EPA Acc. No. 249073.

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DATA EVALUATION RECORD

Citation: Eschbach, J. C., and G. K. Hogan. December 10, 1981. An acute inhalation toxicity study of Terrazole in the rat. Unpublished report no. 3742 prepared by BioDynamics, Inc. Submitted by Olin Corporation. EPA Acc. No. 249073.

Materials and Methods

Test substance: Technical grade Terrazole® was used. The stated purity of the active ingredient was 100%, and it was described as a reddish brown liquid.

Test species: Sprague-Dawley CD rats of both sexes were used. Male rats weighed between 213 and 296 g, and females weighed from 201 to 252 g on the day they were received at the laboratory. They were acclimated for 13 days before exposure to the test substance.

Exposure conditions: The test substance was placed into a 500 ml flask fitted to a fluid metering pump. It was fed to a spray atomizer through which dry air flowed at a rate of 18 liters per minute to produce an aerosol. The generated test atmosphere was directed into a 100 liter exposure chamber containing one group of the test animals.

Four other groups of test animals were exposed to test atmospheres generated with a syringe pump and an atomizer nozzle. Dry air was passed through the atomizer at rates of 2, 4 (for two groups of animals), and 8 liters per minute to generate the test atmospheres to be evaluated.

Nominal exposure concentrations were determined by the difference between the weight of the loaded generator aparatus before each exposure and that of the aparatus after the exposure divided by the amount of air (liters) delivered. Air samples were drawn from the inhalation chamber through paper filters and tandem midget impingers filled with ethylene glycol. (The impingers were not used in sampling of the test atmosphere generated by the first method described above.) The air flow rate through the samplers was 10 liters per minute for a period of 10 minutes for all test atmospheres except the first one described above and the one generated with an atomizer flow rate of 2 liters per minute. The respective conditions for those atmospheres were a duration of 15 seconds

or a duration of 20 minutes. The filter aparatus and paper were weighed before and after the sample, and the difference in the weights was divided by the volume of air sampled to determine test atmosphere concentrations. Filters were also analyzed by gas chromatography. Samples from each end of the test chamber were taken once during the exposure period to determine distribution of the test substance.

Cascade impactors were used to monitor the particle size distribution at 30-minute intervals during the exposure period for most test conditions. Under other conditions (low test concentrations) a Royce Particle monitor was used.

Relative humidity and air temperature in the test chamber were monitored with a wet bulb/dry bulb hygrometer at hourly intervals during the exposure period also.

Experimental procedure: Groups of 5 male and 5 female rats were exposed to each test atmosphere for 4 hours. The animals were observed every 15 minutes during the first hour, hourly during the exposure and for four hours following exposure, and daily for the next 14 days. Signs of toxicity and mortalities were noted. Animals were weighed prior to exposure as well as 1, 2, 4, 7, and 14 days after exposure. After 14 days of observation survivors were sacrificed and necropsied. Those rats dying during the observation period were necropsied as soon after their deaths as possible. The animals were examined grossly and tissue samples of the liver, kidneys, and lungs were prepared for later examination.

The LC_{50} was calculated along with 95% confidence limits by the method of Litchfield and Wilcoxon.

Reported Results

The reported nominal, gravimetric, and analytical results for air concentrations in the test atmospheres are summarized as follows:

Group Designation	Concentrations (mg/l)		
	Nominal	Gravimetric	Analytical
I	67.0	3.4	----
II	11,3	1.0	0.265
V	5,3	0.8	0.068
VII	7.0	0.4	0.033
VIII	2.6	0.06	0.006

The report stated that gravimetric analysis of the control chamber in which a group of rats was exposed to dry air for 4 hours showed an air concentration of 0.11 mg/l, but the gas chromatographic analysis showed no detectable levels of the test substance.

The average aerodynamic mass median diameters reported for test atmospheres for groups I, II, V, VII, and VIII were 3.93, 5.10, 3.34, 4.04, and 3.03 um, respectively. Results with the Royce Particle Monitor for group VIII showed that 99.75% of the particle count had aerodynamic mass diameters of 2 um or less.

The authors noted that all deaths occurred during the five days following exposure. They also stated that there was no apparent dose-related pattern. All 10 animals exposed to each of the two highest nominal air concentrations (67 and 11 mg/l; groups I and II) died, while none of the 10 animals in each of the control and 2.6 mg/l dose group died during the exposure or observation periods. According to the report the calculated LC_{50} was 7.37 mg/l with 95% confidence limits of 4.40 to 12.35. The resulting LC_{50} and 95% confidence limits based on the gravimetric determinations of air concentration were 0.80 and 0.45 to 1.41 mg/l.

During the exposure period the investigators noted the following signs in test animals: lacrimation, salivation, mucoid or red nasal discharge, chromodacryorrhea, closed eyes or swollen eyelids; breathing which was described as irregular, labored, or shallow in animals exposed to the two highest air concentrations; and body tremors, uncoordinated body movement, irregular gait, and convulsions noted in animals exposed to concentrations of 0.8 mg/l or more.

During the 14 days following exposure the authors noted that the neuromuscular and secretory signs described above persisted for 5 to 12 days in survivors. Additional signs observed in animals that died included prostration, general weakness, reduced activity, and signs of eye irritation.

The authors stated that the time of onset, severity, and duration of all of these signs appeared to be dose-related.

Body weights were reported to be decreased for the first 4 days of the observation period in exposed animals, but normal weights and weight gains were reported during the second week after dosing.

In animals examined after spontaneous death, discoloration of the lungs and liver including foci and mottling were noted.

The intensity of these effects was reported to be dose-related according to the report.

In those rats surviving to the end of the study discoloration of the lungs was also noted. The authors stated that the effect was predominant in male rats and indicative of a residual effect.

Discussion and Conclusions

There are adequate data reported to support the conclusion that the LC_{50} for technical grade Terrazole in male and female rats is 7.37 mg/l with 95% confidence limits of 4.40 to 12.35 based on nominal concentration. These data are sufficient to place technical grade Terrazole into Toxicity Category III.

Core Classification: Minimum

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DATA EVALUATION RECORD

NOTE: The following review is adapted from a draft DATA EVALUATION RECORD prepared by MITRE Corporation.

Citation: International Research and Development Corporation. May 27, 1982. Terrazole®: Teratology study in rats. Unpublished report no. 3762. Submitted by Olin Corporation. EPA Acc. No. 249073.

Materials and Methods

Test substance: Technical grade Terrazole (5-ethoxy-3-[trichloromethyl]-1,2,4-thiadiazole) was used. The stated purity was 99%.

Test species: Female COBS CD strain rats were used. Each female was mated overnight with a male and the following morning the vagina of each animal was examined for the presence of a copulatory plug. The day a plug was found was designated Day 0 of gestation. The test animals were approximately 16 weeks of age when mated.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 20 of gestation. Doses of 0, 10, 30, or 75 mg test substance per kg body weight were given to groups of 25 mated dams.

Each dam was observed daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0, 6, 9, 12, 16, and 20 of gestation. Food consumption was not determined in the study. Dosages were adjusted according to the body weights measured on days 6, 9, 12, and 16 of gestation.

The rats were sacrificed on day 20 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and one-half of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and abnormalities were noted.

Statistical procedures are discussed below as apropriate.

Reported Results

The report stated that five high dose group rats died during the study (one each on days 16, 18, and 20 of the study and two on day 19). These animals were observed to have red fluid in the cervix or vagina. The only clinical signs that

were reported in treated groups included alopecia and matting of the hair coats in the anogenital region.

Reported mean maternal body weight for the high dose group was approximately 15% less than that for the control group at days 12, 16, and 20 of gestation. (The body weight at day 20 does not include the gravid uterus.) The group mean body weight gain for the control and high dose groups during the entire study were reported to be 99 and 44 g, respectively.

The reported group mean corpora lutea per dam ranged from 15.8 in the control group to 16.7 in the high dose group. Mean implantations per dam ranged from 13.4 in the low-dose group to 14.1 in the control and high dose groups, and the group mean number of live fetuses per litter ranged from 12.9 in the low-dose group to 13.8 in the high-dose group. None of these three parameters exhibited a relationship to dose.

One dam from the mid-dose group and 2 from the high-dose group were reported to have totally resorbed litters. The mid-dose group dam and one of the high-dose group dams had one implantation site which was lost early in gestation, while the remaining dam in the high-dose group had 15 late and 2 early resorptions. A third dam with 11 live fetuses at termination of the study also had four late resorptions. A Mann-Whitney U test failed to demonstrate statistically significant differences in the incidence of early and late resorptions for the control and high dose groups according to the report ($p>0.05$).

Group mean gravid uterine weights were not reported.

The respective mean fetal weights were 3.6, 3.4, 3.5, and 3.1 g for the control, low, mid, and high dose groups, respectively. The low- and high-dose group means were statistically significantly different from that of the control group ($p<0.05$ and $p<0.01$, respectively).

The overall incidence of fetuses with defects in each group was reported as follows:

Observation	Dose groups			
	Control	Low	Mid	High
	Malformed fetuses			
No. examined	295	332	305	249
With malformations (%)	0 (0)	1 (0.3)	2 (0.7)	3 (1.2)
	Anatomical			
With defects (%)	57 (19)	84 (26)	53 (17)	90 (73)

One fetus from the low dose group was reported to have a testis and bilateral ovaries as well as one uterine horn. There was one case of dyplastic scapula and one case of fused sternebrae reported in the mid-dose group fetuses, and the high-dose group contained two cases of generalized edema (anasarca) and one case of retroesophageal aortic arch.

Most of the anatomical variations reported were in the skeleton and were manifested as delayed ossification in the vertebrae, sternabrae, and bones of the skull. The incidences of the most frequently observed skeletal variations is summarized as follows:

		Dose groups		
Observation	Control	Low	Mid	High
No. examined	145	161	152	125
14th rudimentary rib (%)	24 (16.6)	26 (16.1)	16 (10.5)	31 (24.8)
Unossified sternebrae (%)*	17 (11.7)	36 (22.4)	26 (17.1)	35 (28.0)

*Unossified 5th and/or 6th sternebrae

The percentage of litters containing one or more fetuses with unossified sternebrae for the control, low, mid, and high dose groups was reported to be 54.5, 64.0, 56.5, and 77.8%, respectively.

Although no individual animal data were presented with respect to fetal weights and incidence of skeletal variations, historical control data indicates that those observations in the low-dose group are within laboratory ranges (historical control mean fetal weight is 3.6 g with a range from 3.4 to 4.2 g; the incidence of delayed ossification of sternebrae [reported as above] is 657 of 1441 fetuses examined [45.6%] and ranges from 13 to 75% of the litters with one or more fetuses with the sternebrae effects). The incidence of the skeletal variations in the sternebrae for high-dose group fetuses was slightly above that historically observed according to the report.

The incidence of all other observations of delayed ossification occurred in 6 or fewer fetuses from any given group.

Discussion and Conclusions

The results as presented adequately support the conclusions of the investigators that doses as high as 75 mg/kg/day

administered to pregnant rats did not have teratogenic effects. The decreased maternal body weights, body weight gain, and mortality noted in the dams given the highest dose was indicative of maternal toxicity. The highest dose also caused reduced fetal weights and an increased incidence of delayed ossification (on the basis of total fetuses and litters that contained one or more affected fetuses in each group). Historical control data included in the report also indicated that the decreased fetal body weights and the increased incidence of variations in the sternebrae were within the ranges experienced for the test strain at the test facility.

Based on these considerations the no-observed-effect levels (NOEL) for maternal and fetal toxicity are 30 mg/kg/day, while the lowest-effect levels (LEL) are both 75 mg/kg/day. As noted above, there were no teratogenic effects caused by Terrazole®.

Core Classification: Minimum

DATA EVALUATION RECORD

Citation: Loveday, K. S., and B. A. Donahue. January 26, 1982. In vitro sister chromatid exchange assay on Terrazole®. Unpublished report no. 3745 prepared by Bioassay Systems Corporation. Submitted by Olin Corporation. EPA Acc. No. 249073.

Materials and Methods

Test substance: Technical grade Terrazole was used. The test substance was described as a clear liquid and its composition and purity were not specified. Other substances used in the experiment included dimethylsulfoxide (DMSO) (the vehicle), cyclophosphamide, and mitomycin C. The latter two substances were the reference mutagens.

The metabolic activation system consisted of S9 mixture (Litton Bionetics, lot no. RDL 127).

Test species: Chinese Hamster Ovary (CHO) cells were used as the test organism.

Experimental procedures-cytotoxicity: CHO cells were grown in medium (composition not specified) containing bromodeoxyuridine (BrdU) and concentrations ranging from 0.00001 to 0.5% Terrazole for 30-32 hours (to cell cycles). This experiment was conducted to determine which levels of the test substance in the absence of S9 would permit adequate growth for the assay to detect sister chromatid exchanges (SCE) if they were induced. A similar experiment in which the CHO cells were incubated in medium containing the test substance at the same concentrations for four hours was also conducted. These cultures were then incubated in medium containing BrdU for 30 hours. At the end of the incubation periods for cultures with and without the activation system (S9) cells were harvested and slides were prepared (see below) to determine which concentrations permitted adequate growth for the assay.

SCE assay: The report stated that cells were seeded at 1.0 to 1.5 X 10^6 per culture flask and incubated until the next day. At that time the investigators stated that the medium was removed and replaced with fresh medium containing the test substance at concentrations of 0.001, 0.002, 0.003, 0.004, or 0.005%. Mitomycin C was tested at a concentration of 0.05 ug/ml. The medium also contained BrdU at 2 X 10^{-5} M. These cultures were then incubated for 27.5 hours, and at that time 0.25 ug vinblastin per ml of medium was added. The

cultures were incubated for an additional 2.5 hours.

The cells were then harvested and slides were prepared and stained. The slides were first stained with 33258 Hoechst dye and exposed to ultraviolet light. They were then stained with Giemsa and photographed. The authors stated that approximately equal numbers of metaphase cells from each flask were photographed, and the photographs were scored for SCE's.

Cultures tested in the S9 activation system were handled in a slightly different manner. After the initial seeding and overnight incubation (described above) the cells were resuspended in serum free medium containing the same concentrations of test substance as used in the non-activated test system (described above). A 10% solution of S9 mix (1 ml with 9 ml cofactor solution, composition unspecified) was added to each culture flask (0.5 ml aliquot). These cultures were incubated, cells harvested, slides prepared, and metaphase cells scored in the same manner as those described above in the non-activated phase of the assay.

Reported Results

Cytotoxicity study: The report noted that cncentrations from 0.005 to 0.5% precipitated or prevented the second cell division necessary for the assay. In the activation phase of the preliminary experiment concentrations of 0.006 and 0.007% (highest level tested) were not soluble. All other lower concentrations permitted two cell divisions.

SCE study: The frequency of SCE's per cell (and standard error of the mean) were reported as follows:

Concentration (%)	Number of cells examined	SCE's per cell
Without S9 activation		
0.001	26	13.3 (0.9)
0.002	25	19.8 (1.2)
0.003	22	14.2 (0.9)
0.004	29	17.8 (1.1)
0.005	41	22.7 (1.3)
Mitomycin C	9	54.4 (6.2)
DMSO (vehicle)	22	11.6 (0.9)
Medium	23	16.5 (1.3)

Concentration (%)	Number of cells examined	SCE's per cell
	With S9 activation	
0.001	18	15.2 (1.2)
0.002	26	17.1 (1.0)
0.003	19	17.0 (1.1)
0.004	25	15.6 (1.1)
0.005	24	14.7 (0.8)
Cyclophosphamide + S9	4	120.5 (13.1)
Cyclophosphamide - S9	24	11.6 (1.1)
S9 Mix	27	13.4 (1.1)
DMSO	29	14.0 (1.1)
Medium	27	15.5 (0.9)

The authors noted that an analysis of variance of the results reported from cells without the S9 activation showed a statistically significant increase in the number of SCE's in treated cells (with 99% confidence). A similar analysis of results in the activation phase of the experiment were also statistically significantly increased (with 95% confidence).

Discussion and Conclusions

Although mean frequencies are reported along with ranges and standard deviations, no individual SCE frequencies are reported. This deficiency prevents independent evaluations of the results.

The report also did not include mitotic indeces for each group which could be useful in the interpretation of the reported results with respect to toxicity of the test substance.

Other aspects of the protocol such as specific characterizations of the S9 mix and cofactor solutions used, the purity of the test substance, and details regarding the maintenance of cultures are not reported.

These considerations suggest that the study should be confirmed before a final determination that Terrazole actually significantly increases the frequency of sister chromatid exchanges in CHO cells is made (see DATA EVALUATION RECORD on report no. 3765).

DATA EVALUATION RECORD

Citation: Loveday, K. S. June 4, 1982. The effects of Terrazole® on the In vitro induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. Unpublished report no. 3765 prepared by Bioassay Systems Corporation. Submitted by Olin Corporation. EPA Acc. No. 249073.

Materials and Methods

Test substance: Technical grade Terrazole (99.0% active ingredient) was used. Other substances used in the experiment included dimethylsulfoxide (DMSO) (the vehicle), cyclophosphamide, and mitomycin C. The latter two substances were the reference mutagens.

The metabolic activation system consisted of S9 fraction of livers from Aroclor 1254-treated rats and a cofactor solution which contained $MgCl_2$, KCl, glucose-6-phosphate, NADP, and sodium phosphate buffer (pH 7.4).

Test species: Chinese hamster ovary (CHO) cells were used as the test organism. CHO cells were grown in Ham's F10 medium supplemented with 10% (v/v) newborn calf serum and 50 mg/ml gentamicin. T-25 plastic culture flasks containing 5 ml growth medium were seeded with 1 to $1.5X10^6$ cells and incubated for 20 to 24 hours at 37°. These cultures were reported to be in exponential growth, and were the ones used in the experiments described below.

Experimental procedures-cytotoxicity: The authors noted that the test substance concentrations used in the main study were selected on the basis of preliminary studies described previously (see DATA EVALUATION RECORD on report no. 3745). They also stated that the sister chromatid exchange (SCE) experiment described herein was a followup to the one previously reported.

Chromosomal aberration studies: On the basis of the previous assay (see above) the doses selected for experiments without the S9 activation mixture were 0.002 (in the first test only), 0.003, 0.004, 0.005, and 0.006%, while the assays with the S9 mixture used 0.003, 0.004, 0.005, and 0.006% concentrations.

For assays without S9 mix the cells were incubated in culture medium containing the test substance for 6 hours. At the end of that time vinblastine sulfate (0.25 ug/ml) was added, and the cultures were incubated for another 2 to 3 hours before cells were harvested.

In assays with the S9 mix test cultures were incubated for 2 hours in serum free medium containing the S9 mixture and the test substance. The medium was then replaced with normal medium, and the cultures were incubated for 4 hours followed by treatment with vinblastine sulfate and harvesting as described above.

Metaphase cells were harvested from test cultures by treatment with trypsin. The cells were then concentrated by centrifugation. The cells were then suspended in hypotonic potasium chloride and sodium citrate solution and fixed in methanol/acetic acid. After washings in the fixative, drops of the concentrated suspensions were placed on glass slides and air dried. The slides were then stained with Giemsa stain.

Photomicrographs of metaphase cells were prepared and analyzed for the occurrence of chromosomal breaks, gaps and other aberrations according to the report.

In scoring the number of breaks, the report stated that chromosome breaks and deletions were counted as one break each. Ringed chromosomes, triradials, quadriradials, and dicentrics were counted as two breaks each. The frequency of total breaks per cell was calculated for each test concentration. A positive result was considered to be a frequency which exceeds the upper 95% confidence limit on the historical background frequency and is either twice the concurrent control result or is part of a dose-response relationship which is determined by a least squares plot of the data.

SCE assay: Medium containing the test substance and BrdU at 2×10^{-5} M was inoculated for 27 to 28 hours At the end of that time 0.25 ug vinblastine per ml of medium was added. The cultures were incubated for an additional 2 to 3 hours.

The cells were then harvested and slides were prepared as described above. However, the slides were first stained with 33258 Hoechst dye and exposed to ultraviolet light. The authors stated that approximately equal numbers of metaphase cells from each flask were photographed, and the photographs were scored for SCE's.

Reported Results

Chromosomal aberration assays: According to the report the highest concentration of the test substance (0.006%) induced significant increases in the frequency of chromosomal aberrations in CHO cells without metabolic activation. The investigator noted that the highest concentration caused a

frequency of 0.290 total aberrations per cell compared with 0.017 and 0.052 for the medium and 1% DMSO control groups, respectively. The mitomycin C treated cells exhibited a frequency of 0.350 total aberrations per cell. The results for the first trial are summarized as follows:

Compound and conc. (%)	No. of cells	Chromatid breaks	Other Aberrations	Mitotic index
medium	58	1	none	ND*
1% DMSO	58	3	2 gaps	3.4%
Terrazole				
0.006	62	18	9 gaps	1.1%
0.005	65	9	3 gaps	1.7%
0.005	60	3	2 gaps	2.6%
0.004	60	3	none	4.8%
0.004	62	7	4 gaps	2.3%
0.003	61	2	2 gaps, 1 quadriradial	2.6%
0.003	61	2	1 gap, 1 triradial	2.4%
Mitomycin C	60	16	4 gaps, 2 triradials, 1 deletion	ND*

*ND=not determined

In the second experiment without S9 activation (triplicate cultures for each treatment group) a similarly significant increase in the frequency of aberrations was observed in CHO cells exposed to the 0.005 and 0.006% concentrations. The author noted that the observed average frequency of breaks exceeded the upper 95% confidence limit of 0.100 per cell for historical controls, and the frequencies for the two groups were more than twice that of the negative control group. The reported average frequencies for the 0.005 and 0.006% treatment groups were both 0.103 per cell compared with 0.037 for the concurrent control group. The historical control mean was reported to be 0.034 with a standard deviation of 0.033 and an upper 95% confidence limit of 0.100. The results of this assay are summarized as follows:

Compound and conc. (%)	No. of cells	Chromatid breaks	Other Aberrations	Mitotic index
medium	53	3	2 gaps	ND
	52	0	none	ND
1% DMSO	48	4	1 gap	10.7%
	36	0	none	15.4%
Terrazole				
0.006	53	8	3 gaps	2.8%
0.006	65	10	7 gaps	3.6%
0.006	57	0	3 gaps	3.7%
0.005	56	3	3 gaps	6.3%
0.005	52	10	2 gaps	1.7%
0.005	57	4	1 gap	1.0%
0.004	63	2	3 gaps	ND
0.004	55	4	2 gaps	ND
0.004	56	0	5 gaps	ND
Mitomycin C	32	3	none	ND
	55	4	3 gaps	ND

*ND=not determined

An increase in frequency of aberrations was also noted in cells exposed to the test substance and the S9 activation mixture. The frequencies reported were 0.065, 0.152, 0.064, 0.169, and 0.108 for the negative control, 0.003, 0.004, 0.005, and 0.006% treatment groups, respectively. The author noted that 3 of the 4 test groups exceeded the upper 95% confidence limit for the historical control group (see above), and 2 of those 3 test groups had frequencies that were more than twice that for the concurrent control group indicating a positive response. There was no dose-related increase. Specific results were reported as follows:

Compound and conc. (%)	No. of cells	Chromatid breaks	Other Aberrations	Mitotic index
medium	51	1	1 gap, 1 ring	ND
1% DMSO	47	2	none	4.9%
S9	55	5	3 gaps	ND
Terrazole				
0.006		No metaphase cells		
0.006	81	9	6 gaps	0.5%
0.005		No metaphase cells		

Compound and conc. (%)	No. of cells	Chromatid breaks	Other Aberrations	Mitotic index
Terrazole				
0.005	118	20	12 gaps	1.7%
0.004	68	4	6 gaps	1.1%
0.004	58	4	3 gaps	0.5%
0.003	63	2	5 gaps	1.5%
0.003	62	17	none	1.4%
Cyclophospha-mide	53	8	1 gap	ND

*ND=not determined

SCE study: The frequency of SCE's per cell (and standard error of the mean) were reported as follows:

Concentration (%)	Number of cells examined	SCE's per cell
0.003	29	14.4
0.003	30	18.1
0.004	30	20.8
0.004	20	18.9
0.005	33	15.4
0.005	21	19.7
Mitomycin C	15	66.5
DMSO (vehicle)	24	9.4
	30	10.0
Medium	26	8.3

The authors noted that an analysis of variance of the results showed a statistically significant increase in the number of SCE's in treated cells (with 99% confidence).

Discussion and Conclusions

There were adequate data presented to support the conclusions of the author that Terrazole induces chromosomal aberrations with and without metabolic activation. There was no dose-dependent response noted, but the response was reproducible. The investigator attributed the lack of a dose-related effect to the use of near-toxic concentrations as suggested by the low mitotic indices reported in the chromosomal aberration assays.

An increase in sister chromatid exchanges was also noted to occur in a dose-dependent manner. The results of this assay are similar to those described in a previously reported SCE assay (see DATA EVALUATION RECORD on report no. 3745) indicating that the effect is reproducible.