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ORIGINAL

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Attention: TSCA Section 8(e) Coordinator

Re: Solvay Interlox -- TSCA Section 8(e) -- Peracetic Acid



88950000057

Dear Sir:

This letter and the enclosures are being submitted on behalf of Solvay Interlox ("Interlox"), pursuant to Section 8(e) of the Toxic Substances Control Act, as amended ("TSCA"). Interlox recently received the enclosed unpublished results of a study entitled "The effects of Proxitane-0510 on the chromosomes of cultured human lymphocytes." This study was performed by BIBRA Toxicology International ("BIBRA") with respect to Proxitane-0510. Proxitane-0510 is a compound the primary constituent of which is peracetic acid, CAS No. 79-21-0 ("PAA"). PAA is an oxidizing and corrosive chemical used in disinfectant and sterilization, as a reagent in organic synthesis reaction, and as a biocide. It is not listed as a human carcinogen or potential carcinogen.

The enclosed *in vitro* study results indicated an increased number of aberrant metaphases in human lymphocytes in the absence of metabolic activation. In the presence of metabolic activation, this effect was less pronounced, and only present at toxic concentrations.

In the study, without metabolic activation, evidence of chromosomal aberration was observed in cultured human lymphocytes at concentrations of 1.0 and 1.5 mg/ml (and also at 0.25 and 0.5, but not at 0.75 mg/ml). There were statistically significant increases in the number of aberrant metaphases for all treatments (except 0.5 mg/ml if gaps are excluded), including the positive control (mitomycin C).¹

¹ An "S-9 mix" metabolizing system derived from the livers of rats pretreated with a polychlorinated biphenyl compound (Aroclor) was used, which enhances the activity of drug metabolizing enzymes. Human lymphocytes contain very low levels of the enzymes responsible for the activation of such chemicals, and it is usual to carry out cytogenic studies with these cells both in the presence and absence of an S-9 mix.

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With metabolic activation, effects were observed only at a 5 mg/ml (and possibly 2.5 mg/ml). There were statistically significant increases in the number of aberrant metaphases for treatment with 5 mg/ml and the positive control (cyclophosphamide), but not for treatment at 2.5 or 1.25 mg/ml.

These results indicate that unrealistically high dose levels may contribute to the observed genotoxic response.

In addition to the above study, we are submitting the following negative unpublished studies for a background perspective on PAA:

1. Blowers S.D. (1994) *A micronucleus test with Proxitane-0510*, BIBRA Report No. 1324/1/2/94.
2. Blowers S.D. (1994) *An in vivo unscheduled DNA synthesis assay with Proxitane 0510*, BIBRA Report No. 1434/1/2/94 (only summary available at this time).

In conclusion, the *in vitro* test performed by BIBRA did show an increased number of aberrant metaphases in human lymphocytes in the absence of metabolic activation. In the presence of metabolic activation, this effect was less pronounced and only present at toxic concentrations (5 mg/ml and possibly at 2.5 mg/ml) presumably when the cell protection mechanisms were overcome. However, the results of the positive *in vitro* study should be put into perspective with the negative results of the two enclosed BIBRA *in vivo* studies. In these studies, PAA when administered orally to test animals did not induce micronuclei in the bone marrow in mice nor an increase in unscheduled DNA synthesis in rat hepatocytes. This evidence suggests that PAA is unlikely to represent a genotoxic hazard to man.

Interox wishes to bring the enclosed information to the attention of the Environmental Protection Agency (the "EPA") because it is not aware of any other similar study results for PAA, and the unpublished nature of the information makes it unlikely that the information has otherwise been brought to the EPA's attention.

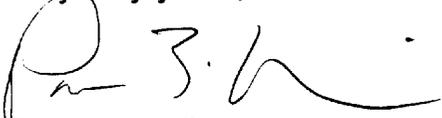
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Please acknowledge your receipt of the enclosures by signing, date stamping and returning the copy of this letter in the enclosed prepaid Federal Express envelope.

If you should have any questions concerning this submission, please do not hesitate to contact the undersigned at (713) 525-6026.

Very truly yours,



Paul J. Harding
Attorney

PJH/jlv

Enclosures



Contains No CEI

The effects of Proxitane-0510 on
the chromosomes of cultured
human lymphocytes

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REPORT No: 1295/1/3/94

PROJECT No: 1295/1

**The effects of Proxitane-0510 on the chromosomes
of cultured human lymphocytes**

BY

B.J. Phillips

DATE OF ISSUE: 7 February 1994

PERFORMING LABORATORY: BIBRA Toxicology International
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BIBRA

STATEMENT OF DATA CONFIDENTIALITY CLAIM

12-02-94 1295/1
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Per: Paul Harding
E.A. Smith

This document contains information confidential and trade secret to Johnson & Johnson Medical Ltd.

It should not be reproduced or microfilmed. It should not be disclosed in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of any other product without the written permission of Johnson & Johnson Medical Ltd.

STATEMENT OF COMPLIANCE

I hereby declare that the study described in this report was conducted under my supervision in compliance with international standards of Good Laboratory Practice and that it constitutes a true record of the actions undertaken and the results obtained.

Study Director:
B.J. Phillips, BSc, PhD, CBiol, MIBiol.

Signature..... *B.J. Phillip* Date..... *7th February 1994*

On behalf of BIBRA Management

Director BIBRA Toxicology International:
S.E. Jagers, BSc, PhD, MIBiol.

Signature..... *S.E. Jagers* Date..... *7th February 1994*

Head of Customer & Information Services Division:
P.B. Brantom, BSc, PhD, MIBiol, MCIM.

Signature..... *P.B. Brantom* Date..... *7th February 1994*

This blank page is included in the final report at the request of the sponsor for possible regulatory purposes.

Quality Assurance Programme

The study critical phases were inspected according to the following programme. As far as can be reasonably established, the methods described and the results reported accurately reflect the raw data generated during the study.

<u>Critical Phase</u>	<u>QAU Inspection date</u>	<u>QAU Report to Study Director Acceptance Date</u>	<u>QAU Report Management Acceptance date</u>
Protocol compliance	11 November 1992	12 November 1992	12 November 1992
Pre-study check	4 December 1992	7 December 1992	7 December 1992
Culture preparation	8 December 1992	8 December 1992	14 December 1992
Test article preparation	6 January 1993	7 January 1993	12 January 1993
Treatment of lymphocytes	27 January 1993	28 January 1993	28 January 1993
Cell harvesting	28 January 1993	28 January 1993	28 January 1993
Preparation of chromosome slides	18 December 1992	18 December 1992	18 December 1992
Assessment			
- mitotic index	26 April 1993	28 April 1993	4 May 1993
- chromosome aberrations	22 March 1993	22 March 1993	22 March 1993

CRITICAL DATES

Preliminary toxicity tests : 8 December 1992-4 January 1993
 Chromosome aberration tests : 4 January 1993-13 May 1993

Data/report audits

The data for this study and the draft and final reports were audited between 2 March 1993 and 7 February 1994. Audit reports were accepted by the Study Director between 17 March 1993 and 7 February 1994 and by BIBRA Toxicology International Management between 18 March 1993 and 7 February 1994.

Archives

All raw data, documentation, any relevant specimens and a copy of the protocol and final report will be retained, for a period of 10 years, in the BIBRA Toxicology International archives under the appropriate reference. Specimens will be retained as long as they afford evaluation.

Protocol deviations

One deviation from the final protocol was noted during the conduct of the study:

In the second chromosome aberration study with metabolic activation, the concentration of the positive control was only 8 $\mu\text{g}/\text{ml}$, not 10 $\mu\text{g}/\text{ml}$ as stated in the study protocol.

As discussed in the text of the report, in the opinion of the Study Director this deviation did not affect the outcome of the study.

Quality Assurance
P.B. Ellis, BSc.

Signature..... Paul B. Ellis..... Date..... 8th February 1994.....

The following contributed to this report in the capacities indicated.

Responsible staff

Study Director	:	Anne J. Edwards, BSc, PhD, CBiol, MIBiol. (from 11 November 1992 to 3 August 1993)
	:	B.J. Phillips, BSc, PhD, CBiol, MIBiol. (from 4 August 1993)
Statistician	:	D.P. Lovell, BSc, PhD, FSS, MIBiol.
Quality Assurance	:	P.B. Ellis, BSc.
Head of Customer & Information Services Division	:	P.G. Brantom, BSc, PhD, MIBiol, MCIM.
Director	:	S.E. Jagers, BSc, PhD, MIBiol.

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SUMMARY

Proxitane-0510 was evaluated for its potential to induce chromosome aberrations in cultured human lymphocytes.

The test article was soluble in culture medium up to the arbitrary maximum of 5 mg/ml defined in the protocol.

Human lymphocyte cultures were exposed to the test article either continuously in the absence of S-9 mix or for 3 h in the presence of S-9 mix, containing post-mitochondrial supernatant from the liver of Aroclor-treated rats. Preliminary studies showed that the mitotic index was not inhibited over the 20 h period following addition of the test article at concentrations up to 0.5 mg/ml in the absence or presence of S-9 mix. Mitotic index was inhibited at 5 mg/ml giving values of 0% and 57.5% of control in the absence or presence of S-9 mix respectively.

Initially, two chromosome aberration tests were conducted, each employing cultures treated in duplicate with a range of 5 concentrations of the test article together with untreated and positive controls. One test was carried out without S-9 mix, treating the cells at 0.25, 0.5, 1, 2 and 4 mg/ml for 20 h. The other test was carried out in the presence of S-9 mix at 0.31, 0.63, 1.25, 2.5 and 5 mg/ml, with treatment limited to 3 h. In both tests, cells were treated 48 h after culture initiation. The cells were arrested in metaphase and harvested 20 h after the start of treatment. Slides were prepared and scored for mitotic index. Test article treatments at 4 and 2 mg/ml reduced the mitotic index of the cells to below 25% of the control in the test without S-9 mix, so chromosome analysis was conducted on the three lowest concentrations and the controls. In the test with S-9 mix, the highest concentration of test article reduced the mitotic index to 69% and chromosome analysis was therefore conducted on the three highest concentrations and the controls. There were statistically significant increases in the number of aberrant metaphases for all treatments (except 0.5 mg/ml if gaps were excluded), including the positive control (mitomycin C), without S-9 mix. With S-9 mix, there were statistically significant increases in the number of aberrant metaphases for treatment with 5 mg/ml and the positive control (cyclophosphamide) but not for treatment at 2.5 or 1.25 mg/ml.

The chromosome aberration tests were repeated using the same treatments for the test in the presence of S-9 mix and a lower range for the test in the absence of S-9 mix. Cultures were included for harvest and chromosome aberration evaluation at 44 h after treatment. At the 20 h harvest, a statistically significant increase in the number of aberrant metaphases, both including and excluding gaps, was induced by the two highest concentrations of test article (1.5 and 1.0 mg/ml) without S-9 mix. With S-9 mix, there was a statistically significant increase, both including and excluding gaps, at the highest concentration (5 mg/ml) and an increase at the second highest concentration (2.5 mg/ml) only if gaps were included.

These statistically significant effects were considered to represent a true clastogenic effect under the conditions employed in the test. Both positive controls were significantly positive. At the 44 h harvest, chromosome analysis was conducted only on the untreated controls and test article concentrations of 1.5 mg/ml without S-9 mix and 5.00 mg/ml with S-9 mix. A statistically significant effect was found for both test article treatments.

It was concluded that, under the conditions employed, Proxitane-0510 causes chromosome damage in cultured human lymphocytes at concentrations of 1.0 and 1.5 mg/ml (and also at 0.25 and 0.5, but not at 0.75 mg/ml) without metabolic activation and at 5 mg/ml (and possibly 2.5 mg/ml) with metabolic activation.

1. INTRODUCTION AND BACKGROUND

Tests for chromosome aberration in mammalian cells are widely used in the evaluation of the potential of chemicals to cause mutations. The presence of chromosome aberrations in treated cells is visible evidence of genetic damage, primarily breakage of DNA molecules. A wide variety of mutagenic and carcinogenic chemicals has been shown to induce chromosome aberrations (Dean & Danford, 1984; Evans & O'Riordan, 1975; Hsu *et al.*, 1977; Ishidate, 1988) and the value of the technique for detecting carcinogens has been reviewed by Preston *et al.* (1981). The cells most commonly used for chromosome aberration tests *in vitro* are human lymphocytes and Chinese hamster cell lines. In the current study human lymphocytes have been employed.

The basis of the test is to expose growing cells to a range of concentrations of the test article and to arrest cell division in the metaphase stage at one or more harvest times after treatment. Stained metaphase preparations are examined for chromosome aberrations. There is some controversy over the biological significance of one category of chromosome aberrations, namely gaps (Anderson & Richardson, 1981), and for this reason data is presented both including and excluding gaps.

Many chemicals are genotoxic only after metabolic activation. Human lymphocytes contain very low levels of the enzymes responsible for the activation of such chemicals and it is usual to carry out cytogenetic studies with these cells both in the presence and absence of a metabolising system (S-9 mix) derived from the livers of rats pretreated with a polychlorinated biphenyl compound (Aroclor) which enhances the activity of the drug-metabolising enzymes. This procedure was adopted in the present study.

The basic sensitivity of the test system was evaluated using a positive control mitomycin C which does not require metabolic activation. The activity of the metabolising system was assessed using cyclophosphamide which requires metabolic conversion before causing chromosome damage.

The range of concentrations of a chemical which can be studied for chromosomal effects is normally limited by its toxicity. A preliminary study of inhibition of the mitotic index is carried out to establish the maximum concentration which can be applied without inhibiting cell division so much that chromosome analysis is impossible. In the absence of toxicity, the maximum concentration may be limited by solubility or by an arbitrary limit of 5 mg/ml.

The design of the present study was based on the recommendations of the United Kingdom Environmental Mutagen Society Sub-Committee on Guidelines for Mutagenicity Testing (Scott *et al.*, 1990). Thus, quadruplicate cultures were used for the negative controls and duplicate cultures were used for all other treatments. Three concentrations of the test article together with appropriate controls were evaluated for chromosome aberrations 20 h (between 1 and 1.5 population doubling times) after the start of treatment. Tests conducted both with and without S-9 mix were repeated, including additional cultures for chromosome analysis at a later harvest time (44 h after treatment).

Proxitane-0510 is an oxidising and corrosive chemical used in disinfection and sterilisation, as a reagent in organic synthesis reactions and as a biocide. It is not listed as a human carcinogen or potential carcinogen. Currently, there is no evidence indicating mutagenicity and this study was conducted as part of a programme of genotoxicity testing requested by the sponsor.

2. TEST AND CONTROL ARTICLES**2.1. Test article**

The test article, identified as Proxitane-0510, (dated 13 November 1992) specification No. 116016 (peracetic acid 5.17% w/w) was supplied by Interlox Chemicals Ltd, Warrington, Cheshire WA4 6HB. It was a colourless liquid which was soluble in culture medium up to the arbitrary maximum of 5 mg/ml defined in the protocol. The pH of the culture medium was 6.4 after the addition of 5 mg/ml Proxitane-0510, but was unchanged after the addition of 0.5 mg/ml. Although not required by the protocol, the pH of the culture medium was adjusted to that of the control solutions using 0.5 M NaOH, in order to ensure that any effects observed were not due merely to the acidity of the test article solutions. The NaOH did not cause any visible effects on the culture medium/test article solutions. Test solutions were prepared immediately before use.

2.2. Control articles

2.2.1. Mitomycin C, obtained from Sigma Chemical Co. Ltd., (Batch No. 32H0326, acquired 11 August 1992) was used as the positive control article in chromosome aberration tests without metabolic activation. Test solutions were prepared immediately before use.

2.2.2. Cyclophosphamide, obtained from Sigma Chemical Co. Ltd., (Batch No. 70H0948, acquired 11 August 1992) was used as the positive control article in chromosome aberration tests with metabolic activation. Test solutions were prepared immediately before use.

3. TEST SYSTEM**3.1. Human lymphocytes**

Blood was collected from a healthy male volunteer by vene-puncture immediately prior to setting up the cultures. It was thoroughly mixed and heparinised with 52 units/ml lithium heparin.

3.2. Culture medium

The medium used consisted of Eagle's Minimum Essential Medium. This was further supplemented as follows:

	Volume (ml)
- Eagle's Minimum Essential Medium	500
- solution of penicillin (10000 IU/ml) and streptomycin (10000 µg/ml)	2.5
- solution of L-glutamine (200 mM)	5.1
- foetal calf serum (heat inactivated at 55°C for 30 min)	88 (15%)

4. MATERIALS

The materials used in this study and their source were:

Plastic universal bottles (30 ml)	: Bibby Sterilin Ltd, Stone, UK.
Eagle's Minimum Essential Medium	
L-Glutamine solution (200 mM)	
Penicillin & Streptomycin solution	
Plastic centrifuge tubes	
All the above from	: Gibco, Life Technologies Ltd, Paisley, UK
Foetal calf serum	: SeraLab Ltd, Crawley, UK
Phytohaemagglutinin, reagent grade	: Wellcome Diagnostics (Murex Diagnostics Ltd), Dartford, UK
Mitomycin C	
Cyclophosphamide	
Demecolcine	
Lithium heparin	
All the above from	: Sigma Chemical Co. Ltd, Poole, UK
Nicotinamide-adenine dinucleotide phosphate (NADP) disodium salt	
Glucose-6-phosphate, disodium salt	
Both of these from	: Boehringer Mannheim, GmbH W. Germany
Magnesium chloride	
Sodium dihydrogen orthophosphate	
Giemsa stain	
Gurr's buffer	
DPX mountant	
All the above from	: BDH Chemicals Ltd, Poole, UK
Potassium chloride	
di-Sodium hydrogen orthophosphate dihydrate	
Acetic acid glacial (A.R. grade)	
Methanol (A.R. grade)	
All the above from	: FSA Laboratory Supplies, Loughborough, UK
Aroclor 1254	: Monsanto Chemical Co. Ltd., St. Louis, Missouri, USA.

5. PROCEDURES

5.1. Blood culture

For each culture, a 0.8 ml volume of fresh human blood from one volunteer, previously mixed with lithium heparin at approximately 50 units/ml final concentration, was added to a plastic universal bottle containing 10 ml culture medium without serum. Cells were sedimented by centrifugation (170 g, 5 min) and the supernatant withdrawn and discarded. The cell pellet was disrupted and resuspended in 9.5 ml culture medium with 15% foetal calf serum. 100 μ l reconstituted phytohaemagglutinin (90 μ g/ml final concentration) and 100 μ l lithium heparin (20 units/ml final concentration) was added to each culture just prior to incubation at $37 \pm 0.5^\circ\text{C}$ in humidified $5.0 \pm 0.1\%$ CO_2 in air.

5.2. Metabolic activation mixture

Male Sprague-Dawley rats (180-250 g) were given a single i.p. injection of 500 mg/kg Aroclor 1254. After three days they were fasted overnight, killed and the livers homogenised (1 g liver : 3 ml tris/KCl buffer pH 7.4). The homogenate was centrifuged for 20 min at 10000 g and ampoules of the supernatant frozen in liquid nitrogen.

For each test requiring S-9, 1 ml of thawed supernatant was added to 10 ml of a solution containing the following:

NADP	5 mM
Glucose-6-phosphate	15 mM
Potassium chloride	33 mM
Magnesium chloride	8 mM
Phosphate buffer (pH 7.4)	80 mM

The mixture containing S-9 fraction is termed S-9 mix.

5.3. Exposure of cells

All test solutions were prepared immediately before use. Forty-eight hours after initiation cultures were centrifuged and the culture medium removed and replaced with fresh culture medium containing the test

article. The test article and cyclophosphamide were dissolved directly in culture medium. Mitomycin C was dissolved in phosphate buffered saline at 0.5 mg/ml and diluted 1:5000 in culture medium.

In the tests incorporating metabolic activation, S-9 mix was added at the time of treatment to give a final concentration of 10% (v.v) in the test solutions added to the cultures. Because of the toxicity of S-9, treatment in the presence of metabolic activation was terminated after 3 h. Cultures were centrifuged, culture medium containing S-9 mix and test article removed and culture medium without serum added. Cultures were centrifuged again, culture medium without serum removed and culture medium with serum added before reincubation. This is a standard procedure for tests involving S-9, allowing time for activation of the test article, reducing the possibility of toxicity from the S-9 itself, and measuring the required endpoint at a similar time to the parallel test conducted without S-9.

5.4. Preliminary toxicity test

5.4.1. Culture treatment

A series of cultures was set up using heparinised fresh blood, as described in Section 5.1. Cultures were incubated for 48 h before exposure to the test article, as described in Section 6.3.

5.4.2. Mitotic arrest

18 h after the start of treatment, 50 μ l Demecolcine, 10 μ g/ml was added to each culture and the cultures reincubated for 2 h.

5.4.3. Cell harvest

20 h after the start of treatment, cultures were decanted into plastic centrifuge tubes and centrifuged (170 g, 5 min). The culture medium was removed and the cells resuspended in 0.075 M potassium chloride (pre-warmed to 37°C) and left for 5 min. After centrifuging (170 g,

5 min) the potassium chloride was removed and the cells resuspended in fixative (3:1 methanol : acetic acid glacial). The fixative was changed repeatedly until it became clear and the cells stored at approximately -20°C overnight.

5.4.4. Slide preparation

Four slides were prepared from each fixed cell suspension. These were air dried, stained with Giemsa (5% in Gurr's buffer, pH 6.8, 5 min), washed in tap water, rinsed in deionised and filtered water, dried and mounted in DPX. The slides were coded and the code retained by a member of staff not involved in the evaluation of the slides.

5.4.5. Mitotic index evaluation

Slides were scanned with a 40x objective and the number of cells and mitotic figures in each field of view counted and recorded. When a total of 1000 cells had been counted, the percentage of mitotic cells (mitotic index) was calculated for each treatment. The effect of the test article on the mitotic index was estimated by expressing the mitotic index in each treated culture as a percentage of the mitotic index in the untreated control.

5.5. Chromosome aberration test

5.5.1. Culture treatment

A series of cultures was set up using heparinised fresh blood, as described in Section 5.1. Cultures were incubated for 48 h before exposure to the test article as described in Section 5.3.

5.5.2. Mitotic arrest

18 h or 42 h after the start of treatment cells were arrested in metaphase as described in Section 5.4.2.

5.5.3. Cell harvest

20 h or 44 h after the start of treatment cells were harvested as described in Section 5.4.3.

5.5.4. Slide preparation

Four slides were prepared and stained as described in Section 5.4.4. (except 4 mg/ml culture in the first chromosome aberration test without S-9 mix : 2 slides only, due to a technical error).

5.5.5. Mitotic index evaluation

Slides were scanned and the mitotic index calculated as described in Section 5.4.5.

5.5.6. Chromosome aberration scoring

Slides were scanned and each metaphase spread examined with a 100x objective. The number of chromosomes in each spread was counted (as the number of centromeres) and those containing 46 centromeres, the normal human complement, were evaluated for aberrations. A total of 100 such metaphases were evaluated for each set of slides.

Aberrations were classified according to the system recommended by the UKEMS guidelines for mutagenicity testing (Scott *et al.*, 1990). For simplicity of presentation, aberrations have been grouped into five categories: gaps, chromatid deletions, chromatid exchanges, chromosome deletions and chromosome exchanges.

The microscope stage vernier co-ordinates were recorded for each metaphase. The number of metaphases showing one or more aberrations both including and excluding gaps as aberrations was calculated for each set of 100.

5.5.7. Statistical analysis

Statistical analysis was carried out following the recommendations of the UKEMS sub-committee on guidelines for mutagenicity testing (Richardson *et al.*, 1989). The data analysed were the proportions of aberrant metaphases in each sample, both including and excluding gaps as aberrations.

5.5.7.1. Tests for homogeneity

The results of each chromosome aberration test (excluding the positive controls) were tested for homogeneity between slides within treatments using the heterogeneity chi-squared test (Kirkland, 1989). There was no evidence of heterogeneity in either of the tests carried out in the absence of S-9 mix or in the second test in the presence of S-9 mix and so the results for each pair of duplicate cultures (or quadruplicate cultures for controls) were pooled for further analysis. In the first test carried out in the presence of S-9 mix, some heterogeneity was detected within the control group. This was due to the relatively high level of chromosome aberrations in replicate 4. No attempt was made to correct the Fisher's exact or trend tests to take this heterogeneity into account and results were pooled, as described above. The removal of control replicate 4 from the analysis would have the effect of increasing the levels of significance found in the treated cultures.

5.5.7.2. Test of differences between treated and control cultures

The pooled data for each treatment (number of aberrant metaphases with or without gaps in a sample of 200) were compared with the appropriate control using a one-tailed Fisher exact test, (Sokal & Rohlf, 1969). Positive controls and test article treatments were compared with untreated controls.

The Cochran-Armitage trend test (Gart *et al.*, 1986) was performed in those cases where data were obtained for three concentrations of the test article.

6. STUDY DESIGN

6.1. Preliminary toxicity test

Two sets of 14 cultures were employed. One set was treated for 20 h in the absence of S-9 mix and the other for 3 h in its presence. Cultures from each set were treated in duplicate with each of a series of six test article concentrations from 5 mg/ml to 0.05 μ g/ml, spaced at intervals of 10-fold serial dilution. Two cultures from each set were treated with culture medium only.

Cultures were harvested 20 h after the start of treatment, after mitotic arrest. Slides were made, coded and evaluated for mitotic index.

6.2. Chromosome aberration tests

6.2.1. First chromosome aberration test in the absence of metabolic activation

Cultures were exposed, in duplicate, to test article concentrations of 4, 2, 1, 0.5 and 0.25 mg/ml and to 0.1 μ g/ml mitomycin C (positive control). Four cultures were exposed to culture medium alone (untreated controls). A total of 16 cultures was used. Cultures were harvested 20 h after the start of treatment, after mitotic arrest. Slides were prepared, coded and evaluated for mitotic index. Chromosome analysis was conducted on the slides from all controls and from the three highest concentrations of test article showing a sufficiently high mitotic index i.e. above 25%.

6.2.2. First chromosome aberration test in the presence of metabolic activation

Cultures were exposed in duplicate to test article concentrations of 5, 2.5, 1.25, 0.63 and 0.31 mg/ml and to 10 μ g/ml cyclophosphamide (positive control). Four cultures were exposed to culture medium alone (untreated controls). S-9 mix was included in all treatments. A total of 16 cultures was used. Treatment was for 3 h and cultures were

harvested 20 h after the start of treatment after mitotic arrest. Slides were prepared, coded and evaluated as described in Section 6.2.1.

6.2.3. Second chromosome aberration test in the absence of metabolic activation

Since the mitotic index was extremely low in cultures exposed to test article concentrations of 4 and 2 mg/ml in the first chromosome aberration test in the absence of metabolic activation, these concentrations were not included in the second test. Cultures were exposed as described in Section 6.2.1. to test article concentrations of 1.5, 1, 0.75, 0.5 and 0.25 mg/ml, culture medium alone and mitomycin C.

Although not required by the protocol, a second harvest time was included in the second test for reasons of completeness. Four replicates per treatment were included except for the positive control (2 replicates). Cultures were harvested at 20 h from 2 cultures per treatment (including the positive control) and at 44 h from the remaining cultures. Mitotic index determinations were made only on the 20 h harvest time slides. Chromosome aberrations were scored on the 20 h harvest time slides for all control treatments and three concentrations of test article. For the 44 h harvest only the highest test article concentration giving sufficient metaphases was scored together with the untreated control cultures.

6.2.4. Second chromosome aberration test in the presence of metabolic activation

Cultures were exposed as described in Section 6.2.2. with additional replicates and harvest times as described in section 6.2.3. The positive control, cyclophosphamide, was used at a lower concentration, 8 μ g/ml, due to a dilution error. This deviation from the study protocol is not considered to have affected adversely the outcome of the study.

7. RESULTS

7.1. Preliminary toxicity test

Treatment with the test article had a variable effect on the mitotic index. In the absence or presence of S-9 mix there was evidence of toxicity at 5 mg/ml only (Table 1; Appendix 1). When added to cultures at 0.05, 0.5 or 5 mg/ml, solutions of the test article caused foaming and, at 5 mg/ml only, turned the cultures dark red. It was concluded that chromosome aberration tests could be conducted using 4 mg/ml as the maximum concentration, without S-9 mix and 5 mg/ml with S-9 mix.

7.2. Chromosome aberration tests

7.2.1. First chromosome aberration test in the absence of metabolic activation

Treatment with the test article had a variable effect on the mitotic index (Table 2; Appendix 2). Test article concentrations of 4 and 2 mg/ml reduced the mitotic index to below 25%, so chromosome aberrations were scored for the three lowest concentrations and the controls (Appendix 3).

There were statistically significant increases in aberrant metaphases, including gaps, when the test article concentrations of 1, 0.5 and 0.25 mg/ml were compared with the untreated control; excluding gaps there were significant increases at 1 and 0.25 mg/ml, but not at 0.5 mg/ml (Table 2). There was a statistically significant positive linear trend in the data for test article treatments and the untreated control. Mitomycin C caused a highly significant increase in aberrant metaphases.

7.2.2. First chromosome aberration test in the presence of metabolic activation

Treatment with the test article had a variable effect on the mitotic index (Table 3; Appendix 4). The test article reduced the mitotic index to 69% at 5 mg/ml and chromosome aberrations were scored for the three

highest concentrations and the controls (Appendix 5). There were statistically significant increases in aberrant metaphases, both including and excluding gaps, when a test article concentration of 5 mg/ml was compared with the untreated control, but not for 2.5 or 1.25 mg/ml (Table 3). There was a statistically significant positive linear trend in the data for test article treatments and the untreated control. Cyclophosphamide caused a highly significant increase in aberrant metaphases.

7.2.3. Second chromosome aberration test in the absence of metabolic activation

Treatment with the test article had a variable effect on the mitotic index measured at the 20 h harvest time (Table 4; Appendix 6).

At 1.5 mg/ml the test article reduced the mitotic index to 44.5%, at 1.0 mg/ml to 63.5% and at 0.75 mg/ml to 72.0%. Chromosome aberrations were therefore scored for the three highest concentrations and the controls at the 20 h harvest time (Appendix 7). At the 44 h harvest time, only the untreated controls and the test article concentration of 1.5 mg/ml, i.e. the highest concentration tested, were evaluated for aberrations (Appendix 8) since this was considered adequate for the evaluation of the persistence of the effect.

At the 20 h harvest time there were statistically significant increases in aberrant metaphases, both including and excluding gaps, when test article concentrations of 1.5 or 1 mg/ml were compared with the untreated control, but not for 0.75 mg/ml (Table 4). There was a statistically significant positive linear trend in the data for test article treatments and the untreated control. Mitomycin C caused a highly significant increase in aberrant cells. At the 44 h harvest time, the incidence of aberrant metaphases, both including and excluding gaps, was statistically significantly increased in test article treated cultures compared with the untreated control (Table 5).

7.2.4. Second chromosome aberration test in the presence of metabolic activation

Treatment with the test article had a variable effect on the mitotic index measured at the 20 h harvest time (Table 6; Appendix 9). At 5 mg/ml the test article reduced the mitotic index to 61% and at 2.5 mg/ml to 86.5%. Chromosome aberrations were therefore scored for the three highest concentrations and the controls at the 20 h harvest time (Appendix 10). At the 44 h harvest time, only the untreated controls and the test article concentration of 5 mg/ml, i.e. the highest concentration tested, were evaluated for aberrations (Appendix 11) since this was considered adequate for the evaluation of the persistence of the effect.

At the 20 h harvest time, there were statistically significant increases in aberrant metaphases, both including and excluding gaps, when a test article concentration of 5 mg/ml was compared with the untreated control. For 2.5 mg/ml there was a significant increase only if gaps were included. There was a statistically significant positive linear trend in the data for test article treatments and the untreated control. Cyclophosphamide caused a significant increase in aberrant cells. The degree of increase was lower than in the first test due to the use of a lower concentration of cyclophosphamide (8 μ g/ml instead of 10 μ g/ml). At the 44 h harvest time, the incidence of aberrant metaphases, both including and excluding gaps, was statistically significantly increased in test article treated cultures compared with the untreated control (Table 7).

8. DISCUSSION

The test article showed evidence of toxicity to human lymphocytes at the highest concentrations employed, as indicated by changes in the mitotic index, in the preliminary toxicity tests and in the chromosome aberration tests. Ideally, chromosome aberration tests should be conducted at test article concentrations which show some toxic effect in order to ensure that the maximum possible dose of test article has been used and that an effect on chromosome aberrations has not been missed. In the case of Proxitane-0510, metaphases were scored from cultures treated with a concentration of test article at which some toxic effect was seen.

Both chromosome aberration tests in the absence of metabolic activation and both tests in the presence of metabolic activation showed a statistically significant increase in the number of aberrant metaphases, both including and excluding gaps, at some of the highest test article concentrations for which chromosome aberrations were scored (0.25, 0.5 (including gaps only), 1 and 1.5 mg/ml without metabolic activation; 2.5 (including gaps and in the second test only) and 5 mg/ml with metabolic activation). This was considered to be a true clastogenic effect. Thus, two independent studies of chromosome aberration in the absence and two in the presence of metabolic activation gave evidence of an effect of the test article on the chromosomes of human lymphocytes at 20 h harvest time. In each case, the positive control compounds produced an increase in aberrant metaphases, demonstrating that the tests were able to detect the chromosome damaging effect of known mutagens. The highest dose used at which there was no increase in chromosome aberrations was 0.75 mg/ml without metabolic activation. However, this was in the second test only, and a small increase had been observed at 0.25 and 0.5 mg/ml in the first test where control values were very low. In the presence of metabolic activation, the highest dose at which there was no increase in chromosome aberrations was 1.25 mg/ml, although there was only a small increase in chromosome aberrations at 2.5 mg/ml in only one test and only if gaps were included.

There was also an effect of the highest concentration of the test article at the later harvest time employed in the repeat assay in the absence and in the presence of metabolic activation, demonstrating that the chromosome damaging effect persisted for an additional 24 h. This increase in chromosome aberrations was smaller, and of lower statistical significance, than the increase at the earlier harvest time, indicating that the damage may have been repaired or eliminated to some extent during the longer culture time.

From the results of this study it is concluded that Proxitane-0510 has the capacity to damage human chromosomes under the conditions employed in the tests at concentrations of 1.0 and 1.5 mg/ml (and also at 0.25 and 0.5, but not at 0.75 mg/ml) without metabolic activation and at 5 mg/ml (and possibly 2.5 mg/ml) with metabolic activation.

9. REFERENCES

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Table 1. Results of preliminary toxicity test of Proxitane-0510
on mitotic index in cultured human lymphocytes

Test article concentration $\mu\text{g/ml}$	Without metabolic activation ^a		With metabolic activation (S-9 mix) ^b	
	MI ^c	% control ^d	MI ^c	% control ^d
0	3.45	100.0	2.60	100.0
0.05	2.70	78.5	2.90	117.0
0.50	3.85	121.5	2.40	96.0
5.00	5.60	158.0	2.85	111.5
50.00	4.10	116.5	2.35	92.5
500.00	3.90	110.0	2.45	96.0
5000.00	0.00	0.0	1.45	57.5

MI, mitotic index; ^a, 20 h treatment; ^b, 3 h treatment, 20 h harvest; ^c, mean MI of two replicates (see Appendix 1); ^d, mean % control of two replicates (see Appendix 1).

Table 2. Summary of results of first chromosome aberration test of Proxitane-0510 in the absence of metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index* (% control)	Number of metaphases		Number of aberrant metaphases		Number of aberrations ^b	
			With gaps	Without gaps	With gaps	Without gaps	With gaps	Without gaps
Untreated Control	1	114	100	0	0	0	0	0
	2	86	100	0	0	0	0	0
	Mean	100.0						
Test article 1.00 mg/ml	3	111	100	0	0	0	0	0
	4	89	100	1	1	1	1	1
	Mean	100.0	Total	400	1	1	1	1
Test article 0.50 mg/ml	1	41	100	9	8	11	9	9
	2	53	100	10	8	13	11	11
	Mean	47.0	Total	200	19***	16***	24	20
Test article 0.25 mg/ml	1	104	100	3	2	4	3	3
	2	122	100	2	1	3	1	1
	Mean	113.0	Total	200	5*	3 NS	7	4
Test article 0.10 µg/ml	1	91	100	3	3	3	3	3
	2	127	100	1	1	1	1	1
	Mean	109.0	Total	200	4*	4*	4	4
Mitomycin C 0.10 µg/ml	1	34	100	28	25	37	33	33
	2	71	100	24	21	33	30	30
	Mean	52.5	Total	200	52***	46***	70	63

NS Not significantly greater than control (test article doses and positive controls versus untreated) by Fisher's Exact Test.

* Significantly higher than control ($P \leq 0.05$); *** Significantly higher than control ($P \leq 0.001$).

^a Details of mitotic index are given in Appendix 2.

^b Details of chromosome aberrations are given in Appendix 3.

Table 3. Summary of results of first chromosome aberration test of Proxitane-0510 in the presence of metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index* (% control)	Number of metaphases	Number of aberrant metaphases		Number of aberrations ^b	
				With gaps	Without gaps	With gaps	Without gaps
Untreated Control	1	90	100	0	0	0	0
	2	110	100	1	0	2	0
	Mean	100.0					
Test article 5.00 mg/ml	3	91	100	0	0	0	0
	4	109	100	4	3	4	3
	Mean	100.0	Total	5	3	6	3
Test article 2.50 mg/ml	1	76	100	4	2	10	8
	2	62	100	5	4	9	8
	Mean	69.0	Total	9*	6*	19	16
Test article 1.25 mg/ml	1	106	100	1	0	1	0
	2	76	100	1	0	1	0
	Mean	91.0	Total	2 NS	0 NS	2	0
Cyclophosphamide 10 µg/ml	1	92	100	2	2	2	2
	2	93	100	1	0	1	0
	Mean	92.5	Total	3 NS	2 NS	3	2
Untreated Control	1	54	100	8	5	11	6
	2	68	100	11	8	17	13
	Mean	61.0	Total	19***	13***	28	19

NS Not significantly greater than control (test article doses and positive controls versus untreated) by Fisher's Exact Test.

* Significantly higher than control ($P \leq 0.05$).

*** Significantly higher than control ($P \leq 0.001$).

^a Details of mitotic index are given in Appendix 4.

^b Details of chromosome aberrations are given in Appendix 5.

Table 4. Summary of results of second chromosome aberration test of Proxitane-0510 in the absence of metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index* (% control)	Number of metaphases		Total number of aberrant metaphases		Total number of aberrations ^b	
			With gaps	Without gaps	With gaps	Without gaps	With gaps	Without gaps
Untreated Control	1	100	100	3	1	3	1	
	2	100	100	3	3	3	3	
	Mean	100.0						
Test article 1.50 mg/ml	3	111	100	2	1	2	1	
	4	89	100	1	1	1	1	
	Mean	100.0	Total	9	6	9	6	
Test article 1.00 mg/ml	1	43	100	8	8	8	9	
	2	46	100	10	6	12	6	
	Mean	44.5	Total	18***	14***	21	15	
Test article 0.75 mg/ml	1	46	100	9	7	12	9	
	2	81	100	8	5	9	5	
	Mean	63.5	Total	17***	12**	21	14	
Mitomycin C 0.10 µg/ml	1	56	100	5	4	5	4	
	2	88	100	4	2	4	2	
	Mean	72.0	Total	9 NS	6 NS	9	6	
Total	1	51	100	19	16	24	18	
	2	33	100	20	13	26	16	
	Mean	42.0	Total	39***	29***	50	34	

NS Not significantly greater than control (test article doses and positive controls versus untreated) by Fisher's Exact Test.

*** Significantly higher than control ($P \leq 0.001$).

* Details of mitotic index are given in Appendix 6.

^b Details of chromosome aberrations are given in Appendix 7.

Table 5. Summary of results of second chromosome aberration test of Proxidane-0510 in the absence of metabolic activation (44 h harvest)

Treatment	Replicate	Number of metaphases	Total number of aberrant metaphases		Total number of aberrations*	
			With gaps	Without gaps	With gaps	Without gaps
Untreated Control	1	100	2	1	2	1
	2	100	0	0	0	0
	3	100	3	2	3	2
	4	100	5	2	5	2
	Total	400	10	5	10	5
Test article 1.50 mg/ml	1	100	8	6	10	8
	2	100	4	4	5	5
	Total	200	12*	10**	15	13

* Significantly higher than control (P<0.05).

** Significantly higher than control (P<0.01).

Details of chromosome aberrations are given in Appendix 8.

(test article versus untreated) by Fisher's Exact Test.

Table 6. Summary of results of second chromosome aberration test of Proxitang-0510 in the presence of metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index* (% control)	Number of metaphases	Total number of aberrant metaphases		Total number of aberrations ^b	
				With gaps	Without gaps	With gaps	Without gaps
Untreated Control	1	90	100	2	2	3	3
	2	110	100	1	1	1	1
	Mean	100.0					
	3	116	100	3	2	3	2
	4	84	100	5	3	5	3
	Mean	100.0	Total	11	8	12	9
Test article 5.00 mg/ml	1	63	100	16	6	20	8
	2	59	100	17	13	22	18
	Mean	61.0	Total	33***	19***	42	26
Test article 2.50 mg/ml	1	63	100	6	3	7	3
	2	110	100	6	5	6	5
	Mean	86.5	Total	12*	8 NS	13	8
Test article 1.25 mg/ml	1	119	100	3	1	3	1
	2	-	0 ^c	-	-	-	-
	Mean	119.0	Total	3 NS	1 NS	3	1
Cyclophosphamide 8 µg/ml	1	38	100	9	4	10	5
	2	80	100	8	7	10	8
	Mean	59.0	Total	17**	11*	20	13

NS Not significantly greater than control (test article doses and positive controls versus untreated) by Fisher's Exact Test.

* Significantly higher than control ($P \leq 0.05$); ** Significantly higher than control ($P \leq 0.01$)

*** Significantly higher than control ($P \leq 0.001$).

^a Details of mitotic index are given in Appendix 9.

^b Details of chromosome aberrations are given in Appendix 10.

^c For technical reasons, metaphases could not be scored from this culture.

Table 7. Summary of results of second chromosome aberration test of Proxitane-0510 in the presence of metabolic activation (44 h harvest)

Treatment	Replicate	Number of metaphases	Total number of aberrant metaphases		Total number of aberrations*	
			With gaps	Without gaps	With gaps	Without gaps
Untreated Control	1	100	0	0	0	0
	2	100	2	1	2	1
	3	100	2	0	2	0
	4	100	1	1	1	1
	Total	400	5	2	5	2
Test article 5.00 mg/ml	1	100	7	4	11	6
	2	100	3	2	3	2
	Total	200	10**	6*	14	8

* Details of chromosome aberrations are given in Appendix 11.

* Significantly higher than control ($P \leq 0.05$).

** Significantly higher than control ($P \leq 0.01$).

(test article versus untreated) by Fisher's Exact Test.

Appendix 1. Mitotic index from preliminary toxicity test of Proxitane-0510

Test article concentration $\mu\text{g/ml}$	Replicate	Without metabolic activation ^a		With metabolic activation (S-9 mix) ^b	
		MI	% control	MI	% control
0	1	2.7	100	2.9	100
	2	4.2	100	2.3	100
	Mean	3.45	100.0	2.60	100.0
0.05	1	2.1	78	2.0	69
	2	3.3	79	3.8	165
	Mean	2.70	78.5	2.90	117.0
0.50	1	4.5	167	1.9	66
	2	3.2	76	2.9	126
	Mean	3.85	121.5	2.40	96.0
5.00	1	3.7	137	2.8	97
	2	7.5	179	2.9	126
	Mean	5.60	158.0	2.85	111.5
50.00	1	2.9	107	2.2	76
	2	5.3	126	2.5	109
	Mean	4.10	116.5	2.35	92.5
500.00	1	2.6	96	2.3	79
	2	5.2	124	2.6	113
	Mean	3.90	110.0	2.45	96.0
5000.00	1	0.0	0	1.3	45
	2	0.0	0	1.6	70
	Mean	0.00	0.0	1.45	57.5

MI, mitotic index; ^a, 20 h treatment; ^b, 3 h treatment, 20 h harvest.

Appendix 2. Mitotic index from first chromosome aberration
test of Proxitane-0510 in the absence of
metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index	
			% control*
Untreated Control	1	7.0	114
	2	5.3	86
	Mean	6.15	100.0
	3	6.1	111
	4	4.9	89
	Mean	5.50	100.0
Test article 1.00 mg/ml	1	2.5	41
	2	2.9	53
	Mean	2.70	47.0
Test article 0.50 mg/ml	1	6.4	104
	2	6.7	122
	Mean	6.55	113.0
Test article 0.25 mg/ml	1	5.6	91
	2	7.0	127
	Mean	6.30	109.0
Mitomycin C 0.10 µg/ml	1	2.1	34
	2	3.9	71
	Mean	3.00	52.5

* The mitotic index for replicate 1 for each treatment was calculated as a percentage of the mean mitotic index for replicates 1 and 2 for control; the mitotic index for replicate 2 for each treatment was calculated as a percentage of the mean mitotic index for replicates 3 and 4 for control.

Appendix 3. Chromosome aberrations found in first chromosome aberration test of Proxirane-0510 in the absence of metabolic activation (20 h harvest)

Treatment	Replicate	Number of metaphases	Total number of aberrations										
			Chromatid					Chromosome					Total
			Gaps	Deletions	Exchanges	Deletions	Exchanges	With gaps	Without gaps				
Untreated Control	1	100	2	0	0	0	0	0	1	0	3	1	
	2	100	0	2	0	0	0	1	0	3	3	3	
	3	100	1	1	0	0	0	0	0	2	1	1	
	4	100	0	0	0	0	0	1	0	1	1	1	
	Total	400	3	3	0	0	0	3	0	9	6	6	
Test article 1.50 mg/ml	1	100	0	3	0	0	0	6	0	9	9	9	
	2	100	6	3	0	0	3	3	0	12	7	7	
	Total	200	6	6	0	0	9	3	0	21	16	16	
Test article 1.00 mg/ml	1	100	3	2	0	0	7	0	0	12	9	9	
	2	100	4	2	1	0	2	0	0	9	5	5	
	Total	200	7	4	1	0	9	0	0	21	14	14	
Test article 0.75 mg/ml	1	100	1	3	0	0	1	0	0	5	4	4	
	2	100	2	0	0	0	2	0	0	4	2	2	
	Total	200	3	3	0	0	3	0	0	9	6	6	
Mitomycin C 0.10 µg/ml	1	100	6	6	2	0	10	0	0	24	18	18	
	2	100	10	8	1	7	0	0	0	26	16	16	
	Total	200	16	14	3	17	0	0	0	50	34	34	

Appendix 4. Mitotic index from first chromosome aberration
test of Proxitane-0510 in the presence of
metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index	
			% control ^a
Untreated Control	1	5.7	90
	2	6.9	110
	Mean	6.30	100.0
	3	5.6	91
	4	6.7	109
	Mean	6.15	100.0
Test article 5.00 mg/ml	1	4.8	76
	2	3.8	62
	Mean	4.30	69.0
Test article 2.50 mg/ml	1	6.7	106
	2	4.7	76
	Mean	5.70	91.0
Test article 1.25 mg/ml	1	5.8	92
	2	5.7	93
	Mean	5.75	92.5
Cyclophosphamide 10 µg/ml	1	3.4	54
	2	4.2	68
	Mean	3.80	61.0

^a The mitotic index for replicate 1 for each treatment was calculated as a percentage of the mean mitotic index for replicates 1 and 2 for control; the mitotic index for replicate 2 for each treatment was calculated as a percentage of the mean mitotic index for replicates 3 and 4 for control.

Appendix 5. Chromosome aberrations found in first chromosome aberration test of Proxitan-0510 in the presence of metabolic activation (20 h harvest).

Treatment	Replicate	Number of metaphases	Number of aberrations								
			Chromatid			Chromosome			Total		
			Gaps	Deletions	Exchanges	Deletions	Exchanges	With gaps	Without gaps		
Untreated Control	1	100	0	0	0	0	0	0	0	0	0
	2	100	2	0	0	0	0	0	0	2	0
	Total	200	2	0	0	0	0	0	0	2	0
Test article 5.00 mg/ml	3	100	0	0	0	0	0	0	0	0	0
	4	100	1	1	0	2	0	0	4	3	0
	Total	200	1	1	0	2	0	0	4	3	0
Test article 2.50 mg/ml	1	100	2	6	0	2	0	0	10	8	0
	2	100	1	4	1	3	0	0	9	8	0
	Total	200	3	10	1	5	0	0	19	16	0
Test article 1.25 mg/ml	1	100	1	0	0	0	0	0	1	0	0
	2	100	1	0	0	0	0	0	1	0	0
	Total	200	2	0	0	0	0	0	2	0	0
Cyclophosphamide 10 µg/ml	1	100	0	2	0	0	0	0	2	2	0
	2	100	1	0	0	0	0	0	1	0	0
	Total	200	1	2	0	0	0	0	3	2	0
Total	1	100	5	5	0	1	0	0	11	6	0
	2	100	4	6	0	7	0	0	17	13	0
	Total	200	9	11	0	8	0	0	28	19	0

Appendix 6. Mitotic index from second chromosome aberration
test of Proxitane-0510 in the absence of
metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index	
			% control*
Untreated Control	1	6.1	100
	2	6.1	100
	Mean	6.10	100.0
	3	6.3	111
	4	5.1	89
	Mean	5.70	100.0
Test article 1.50 mg/ml	1	2.6	43
	2	2.6	46
	Mean	2.60	44.5
Test article 1.00 mg/ml	1	2.8	46
	2	4.6	81
	Mean	3.70	63.5
Test article 0.75 mg/ml	1	3.4	56
	2	5.0	88
	Mean	4.20	72.0
Test article 0.50 mg/ml	1	5.8	95
	2	3.7	65
	Mean	4.75	80.0
Test article 0.25 mg/ml	1	6.0	98
	2	6.1	107
	Mean	6.05	102.5
Mitomycin C 0.10 µg/ml	1	3.1	51
	2	1.9	33
	Mean	2.50	42.0

- * The mitotic index for replicate 1 for each treatment was calculated as a percentage of the mean mitotic index for replicates 1 and 2 for control; the mitotic index for replicate 2 for each treatment was calculated as a percentage of the mean mitotic index for replicates 3 and 4 for control.

Appendix 7. Chromosome aberrations found in second chromosome aberration test of Proxitane-0510 in the absence of metabolic activation (20 h harvest)

Treatment	Replicate	Number of metaphases	Total number of aberrations										
			Chromatid					Chromosome					Total
			Gaps	Deletions	Exchanges	Deletions	Exchanges	With gaps	Without gaps				
Untreated Control	1	100	2	0	0	1	0	0	3	1	0	3	1
	2	100	0	2	0	1	0	0	3	0	0	3	3
	3	100	1	1	0	0	0	0	2	0	0	2	1
	4	100	0	0	0	1	0	0	1	0	0	1	1
	Total	400	3	3	0	3	0	0	9	0	0	9	6
Test article 1.50 mg/ml	1	100	0	3	0	6	0	0	9	0	0	9	9
	2	100	6	3	0	3	0	0	12	0	0	12	6
	Total	200	6	6	0	9	0	0	21	0	0	21	15
Test article 1.00 mg/ml	1	100	3	2	0	7	0	0	12	0	0	12	9
	2	100	4	2	1	2	0	0	9	0	0	9	5
	Total	200	7	4	1	9	0	0	21	0	0	21	14
Test article 0.75 mg/ml	1	100	1	3	0	1	0	0	5	0	0	5	4
	2	100	2	0	0	2	0	0	4	0	0	4	2
	Total	200	3	3	0	3	0	0	9	0	0	9	6
Mitomycin C 0.10 µg/ml	1	100	6	6	2	10	0	0	24	0	0	24	18
	2	100	10	8	1	7	0	0	26	0	0	26	16
	Total	200	16	14	3	17	0	0	50	0	0	50	34

Appendix 8. Chromosome aberrations found in second chromosome aberration test of Proxitan-0510 in the absence of metabolic activation (44 h harvest)

Treatment	Replicate	Number of metaphases	Chromatid					Chromosome					Total
			Gaps	Deletions	Exchanges	Deletions	Exchanges	Deletions	Exchanges	With gaps	Without gaps		
Untreated Control	1	100	1	0	0	0	0	0	1	0	0	2	1
	2	100	0	0	0	0	0	0	0	0	0	0	0
	3	100	1	0	0	0	0	2	0	0	3	2	2
	4	100	3	1	0	0	1	0	1	0	5	2	2
	Total	400	5	1	0	0	4	0	4	0	10	5	5
Test article 1.50 mg/ml	1	100	2	3	1	1	0	4	0	0	10	8	8
	2	100	0	2	0	0	3	0	3	0	5	5	5
	Total	200	2	5	1	1	7	0	7	0	15	13	13

Appendix 9. Mitotic index from second chromosome aberration
test of Proxitane-0510 in the presence of
metabolic activation (20 h harvest)

Treatment	Replicate	Mitotic index	
			% control ^a
Untreated Control	1	5.0	90
	2	6.1	110
	Mean	5.55	100.0
	3	7.7	116
	4	5.6	84
	Mean	6.65	100.0
Test article 5.00 mg/ml	1	3.5	63
	2	3.9	59
	Mean	3.70	61.0
Test article 2.50 mg/ml	1	3.5	63
	2	7.3	110
	Mean	5.40	86.5
Test article 1.25 mg/ml	1	6.6	119
	2 ^b	-	-
	Mean	6.60	119.0
Cyclophosphamide 8 µg/ml	1	2.1	38
	2	5.3	80
	Mean	3.70	59.0

^a The mitotic index for replicate 1 for each treatment was calculated as a percentage of the mean mitotic index for replicates 1 and 2 for control; the mitotic index for replicate 2 for each treatment was calculated as a percentage of the mean mitotic index for replicates 3 and 4 for control.

^b For technical reasons, mitotic index could not be measured for this culture.

Appendix 10. Chromosome aberrations found in second chromosome aberration test of Proxitan-0510 in the presence of metabolic activation (20 h harvest)

Treatment	Replicate	Number of metaphases	Total number of aberrations										
			Chromatid					Chromosome					Total
			Gaps	Deletions	Exchanges	Deletions	Exchanges	With gaps	Without gaps				
Untreated Control	1	100	0	2	0	0	1	0	0	3	0	3	
	2	100	0	1	0	0	0	0	0	1	0	1	
	3	100	1	2	0	0	0	0	0	3	0	2	
	4	100	2	1	0	0	2	0	0	5	0	3	
	Total	400	3	6	0	0	3	0	0	12	0	9	
Test article 5.00 mg/ml	1	100	12	5	0	3	0	0	0	20	0	8	
	2	100	4	10	0	8	0	0	0	22	0	18	
	Total	200	16	15	0	11	0	0	0	42	0	26	
Test article 2.50 mg/ml	1	100	4	0	0	3	0	0	0	7	0	3	
	2	100	1	1	0	4	0	0	0	6	0	5	
	Total	200	5	1	0	7	0	0	0	13	0	8	
Test article 1.25 mg/ml	1	100	2	0	0	1	0	0	0	3	0	1	
	2	0*	-	-	-	-	-	-	-	-	-	-	
	Total	100	2	0	0	1	0	0	0	3	0	1	
Cyclophosphamide 8 µg/ml	1	100	5	1	0	4	0	0	0	10	0	5	
	2	100	2	3	0	5	0	0	0	10	0	8	
	Total	200	7	4	0	9	0	0	0	20	0	13	

* For technical reasons, metaphases could not be scored from this culture.

Appendix 11. Chromosome aberrations found in second chromosome aberration test of Proxidane-0510 in the presence of metabolic activation (44 h harvest)

Treatment	Replicate	Number of metaphases	Total number of aberrations								
			Chromatid				Chromosome				Total
			Gaps	Deletions	Exchanges	Deletions	Exchanges	With gaps	Without gaps		
Untreated Control	1	100	0	0	0	0	0	0	0	0	0
	2	100	1	1	0	0	0	0	2	1	1
	3	100	2	0	0	0	0	0	2	0	0
	4	100	0	0	0	1	0	0	1	1	1
	Total	400	3	1	0	1	0	0	5	2	2
Test article 5.00 mg/ml	1	100	5	2	0	4	0	0	11	6	6
	2	100	1	0	0	2	0	0	3	2	2
	Total	200	6	2	0	6	0	0	14	8	8



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REPORT No: 1434/1/2/94

PROJECT No: 1434/1

An in vivo unscheduled DNA synthesis assay with Proxitane 0510

BY

S.D. Blowers

DATE OF ISSUE: 17 August 1994

PERFORMING LABORATORY: BIBRA Toxicology International
Woodmansterne Road
Carshalton
Surrey
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UK

1. SUMMARY

Proxidane 0510 was assessed for its ability to induce DNA repair in rat hepatocytes, after an oral *in vivo* treatment, using the unscheduled DNA synthesis (UDS) assay.

One study was carried out, in three separate parts, assessing two oral doses of Proxidane 0510 at 0.33 and 1.0 g/kg after two treatment times. No significant increases in UDS, measured as a net grain increase were observed in either of the treated groups and the positive control responses confirmed the validity of the assay.

It was concluded that Proxidane 0510 did not induce unscheduled DNA repair in the *in vivo* UDS assay under the conditions used in the study.



Contains 10 slides

A micronucleus test with
Proxitane-0510

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REPORT No: 1324/1/2/94

PROJECT No: 1324/1

A micronucleus test with Proxitane-0510

BY

S.D. Blowers

DATE OF ISSUE: 7 February 1994

PERFORMING LABORATORY: BIBRA Toxicology International
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STATEMENT OF DATA CONFIDENTIALITY CLAIM.

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STATEMENT OF COMPLIANCE

I hereby declare that the study described in this report was conducted under my supervision in compliance with international standards of Good Laboratory Practice and that it constitutes a true record of the actions undertaken and the results obtained.

Study Director:
S.D. Blowers, BSc, PhD.

Signature..... *S.D. Blowers* Date..... *7th February 1994*

On behalf of BIBRA Management

Director:
S.E. Jagers, BSc, PhD, MIBiol.

Signature..... *S.E. Jagers* Date..... *7th February 1994*

Head of Customer & Information Services Division:
P.B. Brantom, BSc, PhD, MIBiol, MCIM.

Signature..... *P.B. Brantom* Date..... *9th February 1994*

This blank page is included in the final report at the request of the sponsor for possible regulatory purposes.

Quality Assurance Programme

The study critical phases were inspected according to the following programme. As far as can be reasonably established, the methods described and the results reported accurately reflect the raw data generated during the study.

<u>Critical Phase</u>	<u>QAU Inspection date</u>	<u>QAU Report to Study Director Acceptance Date</u>	<u>QAU Report Management Acceptance date</u>
Protocol compliance	2 February 1993	2 February 1993	2 February 1993
Pre-study check			
: preliminary studies	17 March 1993 24 March 1993	18 March 1993 24 March 1993	19 March 1993 24 March 1993
: main study	16 April 1993	16 April 1993	20 April 1993
Test article administration			
: preliminary studies	18 March 1993	18 March 1993	19 March 1993
Test article preparation and administration			
: main study	19 April 1993	20 April 1993	21 April 1993
Necropsy and bone marrow removal } Slide preparation }	21 April 1993	22 April 1993	22 April 1993
Evaluation	12 May 1993	12 May 1993	12 May 1993
<u>Process Inspections</u>			
Animal receipt and randomisation	26 January 1993		28 January 1993
Animal husbandry	9 March 1993		10 March 1993

CRITICAL DATES

Arrival of animals

Preliminary studies	-	11 March 1993 18 March 1993
Main study	-	8 April 1993

Treatment of animals

Preliminary studies	-	18 March-5 April 1993
Main study	-	19 April 1993
24 hour kill	-	20 April 1993
48 hour kill	-	21 April 1993
72 hour kill	-	22 April 1993
Report	-	June 1993

Data/report audits

The data for this study and the draft and final reports were audited between 11 June 1993 and 7 February 1994. Audit reports were accepted by the Study Director between 15 June 1993 and 7 February 1994 and by BIBRA Toxicology International Management between 15 June 1993 and 7 February 1994.

Archives

All raw data, documentation, any relevant specimens and a copy of the protocol and final report will be retained, for a period of 10 years, in the BIBRA Toxicology International archives under the appropriate reference. Specimens will be retained as long as they afford evaluation.

Protocol deviations

A number of protocol deviations were noted during the conduct of the study:

Processing of mouse bone marrow:

- a) (protocol section 6.4.1), a larger volume of foetal calf serum was used.
- b) (protocol section 6.4.2), non-siliconised Pasteur pipettes were used.
- c) (protocol section 6.4.3), in the examination of each bone marrow smear, a maximum of 500 cells were examined, not 1000.
- d) (protocol section 6.4.3), a reduced number of cells were examined from some cyclophosphamide treated animals.

As discussed in the text of the report, in the opinion of the Study Director these deviations did not affect the outcome of the study.

Quality Assurance
P.B. Ellis, BSc.

Signature..... Paul B. Ellis..... Date 3rd February 1994.....

The following contributed to this report in the capacities indicated.

Responsible staff

Study Director	:	S.D. Blowers, PhD.
Deputy Study Director	:	B.J. Phillips, BSc, PhD.
Animal Services	:	S.J. Crees, FIAT.
Statistician	:	D.P. Lovell, BSc, PhD, FSS, MIBiol.
Quality Assurance	:	P.B. Ellis, BSc.
Head of Customer & Information Services Division	:	P.G. Brantom, BSc, PhD, MIBiol, MCIM.
Director	:	S.E. Jagers, BSc, PhD, MIBiol.

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1. SUMMARY

Groups of 15 male and 15 female mice were given a single oral dose of 0 (control), 8, 35 or 150 mg Proxitane-0510/kg bodyweight. A further group was given a single oral dose of 100 mg cyclophosphamide/kg to act as a positive control. Five males and five females from each group were killed at 24, 48 or 72 hr after treatment. At each time point (24, 48 and 72 hr) the femoral bone marrow was removed and examined for the incidence of micronuclei in the polychromatic erythrocytes (PCE), the proportion of polychromatic erythrocytes in the erythrocyte population and the incidence of micronuclei in normochromatic erythrocytes.

There were no significant differences in the frequency of micronuclei in PCE or in NCE between mice treated with Proxitane-0510 and the untreated controls. This was true for all doses of Proxitane-0510 tested, all three sampling times and both sexes of mice. In both male and female mice cyclophosphamide induced a statistically significant increase in micronuclei in PCE at 24 hr and 48 hr and in NCE at 48 hr and 72 hr. This indicates that the system was capable of detecting the effects of a known genotoxin.

Proxitane-0510 did not induce a dose-related decrease in the proportion of PCE, indicating a lack of toxicity of the bone marrow. However, the highest dose tested (150 mg/kg) was found in preliminary studies to be the maximum tolerated dose in both sexes of mice.

The results of this study indicate that Proxitane-0510 does not induce micronuclei in the bone marrow of mice. It can be concluded that Proxitane-0510, given by the oral route, does not cause chromosome damage in the bone marrow of mice and, on this evidence alone, is unlikely to represent a genotoxic hazard to man.

2. INTRODUCTION AND BACKGROUND TO THE STUDY

The objective of this study was to determine the clastogenic potential of Proxitane-0510 *in vivo*, using the mouse micronucleus test, according to OECD guideline No.474 and Annex V to EEC Directive 67/548/EEC as amended by Directive 84/449/EEC, Method B12.

The mouse micronucleus test is widely used as a means of testing chemicals for their ability to induce genetic damage *in vivo*. It is less time consuming than methods involving the detection of chromosome aberrations but employs the same tissue, the bone marrow, and detects similar effects i.e. chromosome breakage and chromosome loss.

The test is based on the detection of micronuclei, which are isolated chromosomes or chromosome fragments enclosed in a membrane. In the bone marrow, micronuclei are detected in polychromatic erythrocytes (PCE) from which the main nucleus has been extruded during the final division of the precursor cell, the normoblast.

Groups of mice are treated with a range of doses of the test article by an appropriate route. Bone marrow is sampled at three time points after treatment (24, 48 and 72 hr) to allow for possible differences in the rate of development of micronuclei induced by different chemicals. In addition to scoring micronuclei in PCE, counts are also made on mature normochromatic erythrocytes (NCE).

As an indication of the toxicity of the test article, counts are made of the numbers of PCE and NCE in the bone marrow samples. Toxicity is indicated by a decrease in the proportion of PCE.

A known genotoxin, in this study cyclophosphamide, is included in the study to demonstrate the sensitivity of the system.

3. TEST AND CONTROL ARTICLES

3.1. Test article

The test article, identified as Proxitane-0510, (dated 13 November 1992) specification No. 116016 (peracetic acid 15.17% w/w) was supplied by Interlox Chemicals Ltd, Warrington, Cheshire WA4 6HB.

Solutions were prepared in saline for dosing of animals. Because a large number of animals were dosed, the time between preparation and administration was up to 2.5 hr. It was considered that the test material would be stable for this period under the conditions of testing (verbal confirmation from Interlox Chemicals Ltd.) but as a precaution, animals were dosed in a random order.

3.2. Control articles

3.2.1. Negative control article

The negative control article was sterile 0.9% saline made in-house using sodium chloride from BDH Ltd, Poole, Dorset, UK.

3.2.2. Positive control article

The positive control article was cyclophosphamide monohydrate supplied by Sigma Chemicals Ltd, Poole, Dorset, UK, (Batch No.70H0948 purchased 11 August 1992). Solutions were prepared in saline (3.2.1.) immediately before dosing the animals.

4. TEST SYSTEM

Male and female mice of the CD-1 derived strain, aged 6-7 weeks were obtained from a barrier-maintained colony (Charles River UK Limited, Margate, Kent). They were kept in the study room for at least 6 days to allow them to acclimatise to study conditions. The mice were housed individually in polypropylene and stainless-steel grid floored cages, suspended over paper for removal of the excreta. Each cage carried a label stating the following:

- BIBRA Project number (1324/1)
- name of Study Director (S.D. Blowers)
- project licence number
- treatment (test article and concentration) of animal contained
- the date of commencement of treatment
- sex of the animal contained
- identification number of the animal contained
- date of necropsy

The racks of cages were kept in a room used solely for the study and controlled to the following conditions:

Temperature	:	19-24°C
Relative humidity	:	45-70%
Lighting	:	artificial, 12 hr light (06.00-18.00 GMT) 12 hr dark (18.00-06.00 GMT)
Air changes	:	Minimum 15/hr with no recirculation using high efficiency filters

The daily records confirmed that the environmental conditions were within the defined ranges throughout the study, except for the final day of the main study when due to an oversight no recordings were made.

The mice were fed a nutritionally adequate diet (R/M1(E) SQC, Special Diets Services Ltd, Witham, Essex) in food hoppers designed for the purpose. Diet from batch No.8687 was used and a copy of the manufacturer's analysis certificate forms part of the study record. Domestic mains tap water was supplied to each cage in a glass bottle fitted with a stainless steel drinking nozzle. Samples of this water are taken from the BIBRA facility at approximately six-monthly intervals and analyzed by the supplying authority. The records of these analyses are kept on a central file.

It was considered that there were no contaminants present in the food or water which could have affected the outcome of the study.

5. PROCEDURES

5.1. Observations

Daily records were kept of the relative humidity and the maximum and minimum temperatures in the animal room. Due to an oversight, no recordings were made on the last day of the main study. It is not considered that this would affect the outcome of the study since the last groups of animals (72 hr examination) were killed on that day. Each day the animals were observed for variations in behaviour or condition. The daily records and the animal data are all retained as part of the study record.

The mice were weighed individually seven and three days before the start of treatment (days -7 and -3), on the day of treatment (day 0) and immediately prior to the designated kill.

5.2. Administration of control and test articles

The positive control and test article solutions were prepared in saline and given by oral intubation on a single occasion to each animal at a dose volume of 10 ml/kg bodyweight.

5.3. Processing of the bone marrow

The mice were killed by cervical dislocation at the appropriate sampling time.

Immediately after killing, both femurs were removed and the marrow aspirated into 5-10 ml foetal calf serum. The cells were then centrifuged at 1000 rpm for 5 minutes and the supernatant removed. The cells in the sediment were carefully mixed by aspiration into a Pasteur pipette and two slides prepared from each sample. The volume of foetal calf serum and the type of pipette used were not as stated in the protocol due to a recent modification of procedure. The changes made are not considered

to have had any effect on the outcome of the study. The slides were air-dried, fixed in methanol and stained with May-Grunwald and Giemsa stains.

5.4. Examination of slides

For each animal, two slides were scored for micronuclei in a total of at least 1000 polychromatic erythrocytes (PCE). The proportion of PCE in the erythrocyte population was determined by counting a total of at least 1000 erythrocytes and the number of normochromatic erythrocytes (NCE) with micronuclei was determined during this count. The number of cells scored was the intended number, the protocol having been rendered ambiguous by a typographical error. In some cases, i.e. animals examined 48 hr or 72 hr after treatment with 100 mg cyclophosphamide/kg there was a severely reduced number of cells present on the slides and it was not possible to find 1000 PCE's or, in some cases, to find 1000 cells in total. A reduced number of cells was scored for these animals. However, since these treatments were positive controls and only 2 time points were affected, it is not considered that the reduction in scored cells had any adverse effect on the outcome of the study.

5.5. Statistical analysis

Body weights of the Proxitane-0510 treated and untreated control animals were compared using analysis of variance and the procedure of Least Significant Difference (LSD). Body weights of untreated controls and cyclophosphamide treated controls were compared using Student's *t*-test. The use of the LSD procedure for the Proxitane-0510 treated groups was considered more appropriate because of the number of treatment levels included.

The data from the micronucleus assay were analysed using a factorial analysis of variance based on the analysis described in Lovell *et al.* (1989)

Statistical analysis of *in vivo* cytogenetic assays in *UKEMS Sub-Committee on Guidelines for Mutagenicity Testing. Part III. Statistical Evaluation of Mutagenicity Test Data* Ed. D.J. Kirkland, Cambridge University Press, pp.184-232. These methods were considered to be more appropriate than t-tests on individual dose levels, and to yield more information.

Separate analyses were carried out for the Proxitane-0510 treated groups and the positive control data. The number of micronucleated PCE and NCE were transformed by taking the square root of the number of micronuclei plus 0.5 to stabilise the variances. The proportion of PCE was calculated as $\frac{PCE}{PCE + NCE}$. These data were not transformed before analysis using the factorial analysis of variance.

The statistical analysis included a comparison of the variability between samples from the same animal (different slides) and variability between animals receiving the same treatment. Comparisons between treated animals and their controls of the same sex and same sampling time were carried out using the LSD test based upon the pooled between animal variability in the factorial design. The tables of means reported show untransformed means but include statistical significances obtained from the analysis of the transformed data.

6. EXPERIMENTAL DESIGN

6.1. Preliminary studies

6.1.1. First preliminary toxicity test

Two male and 2 female mice were treated orally with 200 mg Proxitane-0510/kg. After 1 day, 1 male and 1 female were found to be oedematous and moribund.

6.1.2. Second preliminary toxicity test

Five male and 5 female mice were treated orally with 150 mg Proxitane-0510/kg. All survived for 10 days but signs of toxicity (swollen abdomen in 1 male and 1 female, noisy/laboured breathing in 1 female, piloerection in 4 males and 1 female) were observed during this period. It is considered that 150 mg/kg represents the maximum tolerated dose of Proxitane-0510 by the oral route in male and female mice.

6.2. Micronucleus study

Seventy-five mice of each sex were allocated to treatment groups by use of computer-generated random number tables and each mouse was uniquely identified with an individual ear-punch code, representing its number in the study. The groups and treatments were as follows:

Group	Treatment	Identification Nos. of mice					
		24 hr*		48 hr*		72 hr*	
		M	F	M	F	M	F
1	Control	1- 5	76- 80	26-30	101-105	51-55	126-130
2	Proxitane-0510 8 mg/kg	6-10	81- 85	31-35	106-110	56-60	131-135
3	Proxitane-0510 35 mg/kg	11-15	86- 90	36-40	111-115	61-65	136-140
4	Proxitane-0510 150 mg/kg	16-20	91- 95	41-45	116-120	66-70	141-145
5	Cyclophosphamide 100 mg/kg	21-25	96-100	46-50	121-125	71-75	146-150

M = Male; F = Female

* Planned sampling time

Micronuclei were assessed from all of the groups including all three time points from the positive control.

The mice were treated as in 5.2., killed at the post-treatment times indicated above and processed for evaluation of micronuclei as in 5.3.

7. RESULTS

The preliminary studies showed that the maximum tolerated dose of oral Proxitane-0510 was 150 mg/kg for male and female mice.

In the main study, there were no significant differences in the body weights of control, Proxitane-0510- or cyclophosphamide-treated animals after treatment (Tables 2-3). Two groups of males (Proxitane-0510 150 mg/kg and cyclophosphamide treatment groups) and one group of females (cyclophosphamide treatment group) had significantly lower body weights on day -7. This was not considered to be of any relevance to the outcome of the study.

The incidence of micronuclei and proportion of PCE in the bone marrow are shown in Table 1. Male and female mice given cyclophosphamide showed a statistically significant increase in the frequency of micronucleated PCE at 24 hr and 48 hr. Micronucleated NCE were significantly increased at 48 hr and 72 hr in both male and female mice treated with cyclophosphamide. At all time points cyclophosphamide caused a statistically significant reduction in % PCE, indicating toxicity.

There were no significant differences from control in frequency of micronucleated PCE or NCE in mice given Proxitane-0510 at any dose or any sampling time.

A statistically significant reduction in % PCE was found at 24 hr in male mice treated with Proxitane-0510 at 8 mg/kg, and in female mice 48 hr after treatment with Proxitane-0510 at 150 mg/kg. These effects were small and there was no evidence of a relationship with dose, suggesting that Proxitane-0510 was not significantly toxic to the bone marrow in this study.

8. DISCUSSION

The increase in micronucleated PCE and NCE induced by cyclophosphamide in this assay confirmed the sensitivity of the system. Cyclophosphamide was also toxic to the marrow of the mice as indicated by the reduction in the proportion of young (polychromatic) cells. Proxitane-0510, however, did not induce micronuclei at any of the sampling times or doses employed.

Although there was no clear evidence of toxicity, the highest dose of Proxitane-0510 (150 mg/kg) was the maximum tolerated dose as indicated by preliminary studies. It is considered that the maximum dose used in this study approached the highest possible exposure of the bone marrow via the oral route, consistent with animal survival.

The results of the study indicate that Proxitane-0510 administered orally is not clastogenic to the bone marrow of mice and, on that evidence alone, would not be considered to represent a genotoxic hazard to man.

Table 1. Incidence of micronuclei in the bone marrow of mice given Proxitane-0510.

Treatment	MALE			FEMALE		
	%PCE	%MN in NCE	%MN in PCE	%PCE	%MN in NCE	%MN in PCE
- 24 hour examination -						
Control Saline	65.72 ± 7.052	0	0.22 ±0.130	58.48 ± 4.769	0.10 ±0.135	0.20 ±0.123
Proxitane-0510 8 mg/kg	56.10* ± 5.769	0.14 ±0.225	0.20 ±0.123	65.00 ± 3.402	0	0.16 ±0.167
Proxitane-0510 35 mg/kg	62.04 ± 3.536	0.11 ±0.157	0.16 ±0.089	60.86 ± 6.512	0	0.20 ±0.141
Proxitane-0510 150 mg/kg	59.46 ± 5.825	0.13 ±0.293	0.24 ±0.219	62.22 ± 8.093	0	0.38 ±0.192
Cyclophosphamide 100 mg/kg	47.64*** ±14.068	0.10 ±0.138	3.00*** ±2.083	47.74* ±10.420	0.28 ±0.230	4.00*** ±1.208
- 48 hour examination -						
Control Saline	59.07 ± 3.784	0	0.16 ±0.182	56.89 ± 6.553	0.06 ±0.144	0.08 ±0.110
Proxitane-0510 8 mg/kg	52.94 ± 3.226	0.08 ±0.109	0.22 ±0.084	54.10 ± 4.963	0.09 ±0.119	0.16 ±0.114
Proxitane-0510 35 mg/kg	56.18 ± 3.695	0	0.12 ±0.110	50.20 ± 5.302	0.08 ±0.109	0.22 ±0.130
Proxitane-0510 150 mg/kg	57.51 ± 5.430	0	0.16 ±0.152	47.98* ± 6.568	0.05 ±0.103	0.14 ±0.167
Cyclophosphamide 100 mg/kg	24.23*** ⁴ ±10.964	0.379* ⁴ ±0.291	1.16** ⁴ ±0.791	19.42*** ± 9.53	1.19*** ±0.667	2.87*** ±1.805
- 72 hour examination -						
Control Saline	56.02 ± 6.698	0.05 ±0.116	0.12 ±0.084	50.20 ± 8.077	0.036 ±0.079	0.12 ±0.130
Proxitane-0510 8 mg/kg	57.36 ± 3.052	0.10 ±0.231	0.06 ±0.055	48.96 ± 7.158	0	0.12 ±0.110
Proxitane-0510 35 mg/kg	56.26 ± 4.736	0.10 ±0.222	0.14 ±0.089	50.52 ± 5.671	0.04 ±0.086	0.10 ±0.123
Proxitane-0510 150 mg/kg	55.24 ± 8.261	0.12 ±0.176	0.26 ±0.230	46.02 ± 9.669	0	0.14 ±0.055
Cyclophosphamide 100 mg/kg	5.73*** ⁴ ± 1.294	0.80*** ⁴ ±0.395	0.60 ⁴ ±0.693	10.22*** ± 3.327	0.42** ±0.419	0.75 ±0.929

Individual data are shown in Appendices 1-6.

The values are means and standard deviations from 5 animals unless otherwise indicated by a superscript showing the number of animals.

The groups were compared with the control group using a two-sided least significant difference test (the %MN in NCE data and the %MN in PCE for Proxitane-0510 data were compared with control after transformation by $\sqrt{x + 0.5}$. The %MN in PCE for Cyclophosphamide data were compared with control data after transformation by $\sqrt{((\%MN \text{ in PCE} \times 500) / \text{Total PCE}) + 0.5}$).

Statistically significant differences are denoted by

* p < 0.05, ** p < 0.01, *** p < 0.001

PCE = Polychromatic erythrocytes

NCE = Normochromatic erythrocytes

MN

= Micronuclei

Table 2. Body weights of male mice in a micronucleus assay with Proxitan-0510.

Treatment	Mean body weights (g) \pm SD at time (days)					
	-7	-3	0	1	2	3
- 24 hour examination -						
Control Saline	27.2 ± 0.84	28.8 ± 1.30	28.6 ± 1.52	29.6 ± 1.52		
Proxitan-0510 8 mg/kg	26.0 ± 1.22	27.4 ± 3.97	27.8 ± 1.64	29.4 ± 0.55		
Proxitan-0510 35 mg/kg	26.6 ± 1.14	30.2 ± 1.10	29.8 ± 1.10	30.8 ± 1.92		
Proxitan-0510 150 mg/kg	24.4** ± 1.52	27.2 ± 4.21	27.6 ± 3.97	29.0 ± 1.73		
Cyclophosphamide 100 mg/kg	26.0 ± 1.87	29.2 ± 2.28	29.2 ± 2.59	29.4 ± 2.41		
- 48 hour examination -						
Control Saline	26.6 ± 0.89	29.4 ± 0.89	29.2 ± 0.84		30.6 ± 1.14	
Proxitan-0510 8 mg/kg	27.8 ± 1.48	30.4 ± 1.95	30.2 ± 1.48		31.8 ± 1.30	
Proxitan-0510 35 mg/kg	26.6 ± 1.82	29.4 ± 2.41	28.8 ± 2.28		30.2 ± 2.39	
Proxitan-0510 150 mg/kg	26.6 ± 1.14	29.6 ± 1.14	29.4 ± 1.52		30.6 ± 1.82	
Cyclophosphamide 100 mg/kg	27.4 ± 1.82	30.4 ± 1.14	30.2 ± 1.48		31.4 ± 1.14	
- 72 hour examination -						
Control Saline	28.4 ± 1.52	31.2 ± 1.30	30.8 ± 1.10			32.8 ± 1.64
Proxitan-0510 8 mg/kg	27.0 ± 2.00	29.0 ± 3.24	28.6 ± 3.78			31.0 ± 3.74
Proxitan-0510 35 mg/kg	26.6 ± 1.14	29.0 ± 1.41	28.8 ± 1.30			31.2 ± 1.64
Proxitan-0510 150 mg/kg	28.0 ± 0.00	30.8 ± 0.84	30.8 ± 1.30			32.2 ± 2.05
Cyclophosphamide 100 mg/kg	26.2* ± 1.30	30.0 ± 0.71	29.4 ± 1.14			31.0 ± 1.00

Individual data are shown in Appendix 7.

The Proxitan-0510 treated groups were compared with the control group using a two-sided least significant difference test.

The Cyclophosphamide treated groups were compared with the control group using a two-sided pooled two-sample t-test.

72 hour examination Day -7:- Levels of significance resulting from comparing Control against other groups using data transformed by \log_{10} .

Statistically significant differences are denoted by * $p < 0.05$, ** $p < 0.01$

The values are means and standard deviations from 5 animals.

Table 3. Body weights of female mice in a micronucleus assay with Proxidane-0510.

Treatment	Mean body weights (g) \pm SD at time (days)					
	-7	-3	0	1	2	3
- 24 hour examination -						
Control	23.6	24.4	22.0	24.4		
Saline	± 0.89	± 1.52	± 1.00	± 1.52		
Proxidane-0510 8 mg/kg	23.0	23.8	22.8	25.0		
	± 1.00	± 1.30	± 1.10	± 1.22		
Proxidane-0510 35 mg/kg	23.0	24.4	23.2	25.4		
	± 2.74	± 2.07	± 3.70	± 2.88		
Proxidane-0510 150 mg/kg	22.2	22.8	21.0	22.8		
	± 1.10	± 1.79	± 1.41	± 1.64		
Cyclophosphamide 100 mg/kg	21.6*	23.2	22.4	23.4		
	± 1.52	± 1.64	± 1.14	± 1.14		
- 48 hour examination -						
Control	22.2	23.2	22.8		23.4	
Saline	± 1.64	± 1.79	± 2.05		± 1.67	
Proxidane-0510 8 mg/kg	22.4	22.6	21.6		23.2	
	± 0.55	± 1.34	± 0.89		± 1.10	
Proxidane-0510 35 mg/kg	23.0	24.6	23.6		25.4	
	± 1.58	± 1.67	± 1.34		± 1.95	
Proxidane-0510 150 mg/kg	22.2	23.4	22.2		23.2	
	± 1.30	± 1.52	± 1.30		± 1.64	
Cyclophosphamide 100 mg/kg	23.0	23.4	22.4		24.6	
	± 1.22	± 2.30	± 2.19		± 2.19	
- 72 hour examination -						
Control	22.4	23.0	22.6			23.6
Saline	± 0.55	± 0.71	± 0.89			± 1.82
Proxidane-0510 8 mg/kg	22.2	24.2	23.8			24.6
	± 1.92	± 3.42	± 3.27			± 3.36
Proxidane-0510 35 mg/kg	22.2	23.0	21.8			24.0
	± 1.64	± 2.24	± 1.64			± 2.24
Proxidane-0510 150 mg/kg	22.6	23.2	21.8			23.2
	± 0.55	± 0.84	± 0.84			± 1.30
Cyclophosphamide 100 mg/kg	22.2	22.6	22.2			23.0
	± 0.84	± 1.52	± 1.30			± 1.87

Individual data are shown in Appendix 8.

The Proxidane-0510 treated groups were compared with the control group using a two-sided least significant difference test.

The Cyclophosphamide treated groups were compared with the control group using a two-sided pooled two-sample t-test.

Statistically significant differences are denoted by * $p < 0.05$

The values are means and standard deviations from 5 animals.

Appendix 1. Individual incidences of micronuclei in the bone marrow of male mice in a study with Proxitan-0510 - 24 hour examination.

Treatment and Animal Number	Slide A					Slide B					Combined values				
	PCE	NCE	MN in NCE	Total PCE	MN in PCE	PCE	NCE	MN in NCE	Total PCE	MN in PCE	PCE	NCE	MN in NCE	Total PCE	MN in PCE
<u>Control</u>															
<u>Saline</u>															
1	312	188	0	500	0	258	242	0	500	0	570	430	0	1000	0
2	325	175	0	500	3	331	169	0	500	0	656	344	0	1000	3
3	378	122	0	500	1	389	111	0	500	1	767	233	0	1000	2
4	302	198	0	500	2	343	157	0	500	1	645	355	0	1000	3
5	323	177	0	500	3	325	175	0	500	0	648	352	0	1000	3
<u>Proxitan-0510</u>															
<u>8 mg/kg</u>															
6	243	257	1	500	0	247	253	0	500	2	490	510	1	1000	2
7	279	221	1	500	1	333	167	1	500	2	612	388	2	1000	3
8	298	202	0	500	0	303	197	0	500	0	601	399	0	1000	0
9	248	252	0	500	2	259	241	0	500	1	507	493	0	1000	3
10	299	201	0	500	0	296	204	0	500	2	595	405	0	1000	2
<u>Proxitan-0510</u>															
<u>35 mg/kg</u>															
11	311	189	0	500	0	299	201	0	500	2	610	390	0	1000	2
12	333	167	1	500	1	348	152	0	500	0	681	319	1	1000	1
13	308	192	1	500	1	301	199	0	500	0	609	391	1	1000	1
14	306	194	0	500	1	308	192	0	500	0	614	386	0	1000	1
15	299	201	0	500	1	289	211	0	500	2	588	412	0	1000	3
<u>Proxitan-0510</u>															
<u>150 mg/kg</u>															
16	319	181	0	500	0	273	227	0	500	1	592	408	0	1000	1
17	289	211	2	500	2	270	230	0	500	1	559	441	0	1000	3
18	240	260	2	500	1	302	198	1	500	0	542	458	3	1000	1
19	277	223	0	500	2	311	189	0	500	4	588	412	0	1000	6
20	331	169	0	500	0	361	139	0	500	1	692	308	0	1000	1
<u>Cyclophosphamide</u>															
<u>100 mg/kg</u>															
21	278	222	0	500	1	335	165	1	500	2	613	387	1	1000	3
22	294	206	1	500	9	297	203	0	500	3	591	409	1	1000	12
23	134	366	0	500	25	128	372	0	500	22	262	738	0	1000	47
24	259	241	0	500	19	218	282	0	500	24	477	523	0	1000	43
25	218	282	0	500	22	221	279	0	500	23	439	561	0	1000	45

MN = Micronuclei

PCE = Polychromatic erythrocytes

NCE = Normochromatic erythrocytes

Appendix 2. Individual incidences of micronuclei in the bone marrow of male mice in a study with Proxitan-0510 - 48 hour examination.

Treatment and Animal Number	Slide A				Slide B				Combined values					
	PCE	NCE	MN in NCE	Total PCE	MN in NCE	Total PCE	MN in NCE	Total PCE	PCE	NCE	MN in NCE	Total PCE	MN in NCE	Total PCE
<u>Control</u>														
<u>Saline</u>														
241	259	0	500	3	308	192	0	500	0	549	451	0	1000	3
320	180	0	500	0	286	214	0	500	0	606	394	0	1000	0
27	204	0	500	0	267	233	0	500	1	563	437	0	1000	1
28	311	0	500	0	279	221	0	500	0	590	410	0	1000	1
29	323	0	500	2	323	178	0	500	2	646	355	0	1000	4
30														
<u>Proxitan-0510</u>														
<u>8 mg/kg</u>														
260	240	1	500	1	230	270	0	500	1	490	510	1	1000	2
31	217	0	500	0	265	235	0	500	2	548	452	0	1000	2
32	245	0	500	1	260	240	1	500	0	505	495	1	1000	1
33	257	0	500	2	277	223	0	500	1	534	466	0	1000	3
34	276	0	500	2	294	206	0	500	1	570	430	0	1000	3
35														
<u>Proxitan-0510</u>														
<u>35 mg/kg</u>														
289	211	0	500	0	275	225	0	500	0	564	436	0	1000	0
36	249	0	500	0	307	233	0	500	1	516	484	0	1000	1
37	281	0	500	0	307	193	0	500	3	588	412	0	1000	3
38	295	0	500	1	311	189	0	500	0	606	394	0	1000	1
39	248	0	500	0	287	213	0	500	1	535	465	0	1000	1
40														
<u>Proxitan-0510</u>														
<u>150 mg/kg</u>														
266	234	0	500	0	309	191	0	500	1	575	425	0	1000	1
41	265	0	500	3	281	219	0	500	1	546	454	0	1000	4
42	236	0	500	1	271	230	0	500	0	507	494	0	1000	1
43	353	0	500	2	298	202	0	500	0	651	349	0	1000	2
44	335	0	500	0	262	238	0	500	0	597	403	0	1000	0
45														
<u>Cyclophosphamide</u>														
<u>100 mg/kg</u>														
120	380	1	500	3	123	377	3	500	6	243	757	4	1000	9
46	75	1	250	2	177	323	4	500	6	252	748	5	750	8
47	174	0	250	5	197	303	2	500	12	371	629	2	750	17
48	25	0	125	0	78	422	0	125	1	103	897	0	250	1
49														
50														

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes

MN = Micronuclei
'-' Value unobtainable

Appendix 3. Individual incidences of micronuclei in the bone marrow of male mice in a study with Proxitan-0510 - 72 hour examination.

Treatment and Animal Number	slide A				slide B				Combined values						
	PCE	NCE	MN in Total PCE	MN in PCE	PCE	NCE	MN in Total PCE	MN in PCE	PCE	NCE	MN in Total PCE	MN in PCE			
<u>Control</u>															
<u>Saline</u>															
51	308	192	0	500	2	309	191	0	500	0	617	383	0	1000	2
52	227	273	0	500	1	247	253	0	500	1	474	526	0	1000	2
53	318	182	1	500	1	297	203	0	500	0	615	385	1	1000	1
54	243	257	0	500	0	355	155	0	500	1	598	412	0	1000	1
55	246	254	0	500	0	257	243	0	500	0	503	497	0	1000	0
<u>Proxitan-0510</u>															
<u>8 mg/kg</u>															
56	308	192	0	500	0	305	195	2	500	1	613	387	2	1000	1
57	276	224	0	500	0	254	246	0	500	0	530	470	0	1000	0
58	288	212	0	500	1	275	225	0	500	0	563	437	0	1000	1
59	311	189	0	500	0	275	225	0	500	0	586	414	0	1000	0
60	292	208	0	500	1	284	216	0	500	0	576	424	0	1000	1
<u>Proxitan-0510</u>															
<u>35 mg/kg</u>															
61	316	184	2	500	2	281	219	0	500	0	597	403	2	1000	2
62	263	237	0	500	0	290	210	0	500	0	553	447	0	1000	0
63	267	233	0	500	1	252	248	0	500	1	519	481	0	1000	2
64	304	196	0	500	1	321	179	0	500	1	625	375	0	1000	2
65	257	243	0	500	0	262	238	0	500	1	519	481	0	1000	1
<u>Proxitan-0510</u>															
<u>150 mg/kg</u>															
66	282	218	2	500	4	193	307	0	500	1	475	525	2	1000	5
67	293	207	0	500	1	329	171	0	500	1	622	378	0	1000	2
68	235	265	0	500	0	221	279	0	500	1	456	544	0	1000	1
69	297	203	1	500	5	278	222	0	500	0	575	425	1	1000	5
70	309	191	0	500	0	325	175	0	500	0	634	366	0	1000	0
<u>Cyclophosphamide</u>															
<u>100 mg/kg</u>															
71	19	481	0	125	1	39	461	3	125	0	58	942	3	250	1
72	16	484	3	125	3	40	456	4	125	1	40	960	7	250	4
73	31	469	4	125	0	24	476	4	125	1	71	925	8	250	1
74	36	464	2	125	0	24	476	10	125	0	60	940	12	250	0
75 *	17	483	1	125	0	-	-	-	-	-	-	-	-	-	-

MN = Micronuclei
 '-' = Value unobtainable

PCE = Polychromatic erythrocytes
 NCE = Normochromatic erythrocytes

* = Data not included in statistical analysis.

Appendix 4. Individual incidences of micronuclei in the bone marrow of female mice in a study with Proxitan-0510 - 24 hour examination.

Treatment and Animal Number	Slide A				Slide B				Combined values			
	PCE : NCE	MN in NCE	Total PCE	MN in PCE	PCE : NCE	MN in NCE	Total PCE	MN in PCE	PCE : NCE	MN in NCE	Total PCE	MN in PCE
<u>Control</u>												
<u>Saline</u>												
76	254	246	500	2	313	187	500	2	567	433	1000	4
77	279	221	500	0	300	200	500	1	579	421	1000	1
78	272	228	500	0	246	254	500	1	518	482	1000	1
79	305	195	500	1	315	185	500	1	620	380	1000	2
80	321	179	500	1	319	181	500	1	640	360	1000	2
<u>Proxitan-0510</u>												
<u>8 mg/kg</u>												
81	317	183	500	1	358	142	500	1	675	325	1000	2
82	333	167	500	3	333	167	500	1	666	334	1000	4
83	270	230	500	1	349	151	500	1	619	381	1000	2
84	350	150	500	0	332	168	500	0	682	318	1000	0
85	301	199	500	0	307	193	500	0	608	392	1000	0
<u>Proxitan-0510</u>												
<u>35 mg/kg</u>												
86	344	156	500	1	307	193	500	1	651	349	1000	2
87	297	203	500	2	294	206	500	0	591	409	1000	2
88	321	179	500	2	301	199	500	2	622	378	1000	4
89	350	150	500	1	323	177	500	1	673	327	1000	2
90	247	253	500	0	259	241	500	0	506	494	1000	0
<u>Proxitan-0510</u>												
<u>150 mg/kg</u>												
91	257	243	500	3	255	245	500	1	512	488	1000	4
92	369	131	500	1	326	174	500	0	695	305	1000	1
93	300	200	500	3	297	203	500	2	597	403	1000	5
94	350	150	500	0	359	141	500	3	709	291	1000	3
95	281	219	500	4	317	183	500	2	598	402	1000	6
<u>Cyclophosphamide</u>												
<u>100 mg/kg</u>												
96	241	259	500	27	399	101	500	19	640	360	1000	46
97	210	290	500	24	206	294	500	28	416	584	1000	52
98	303	197	500	15	212	288	500	27	515	485	1000	42
99	165	335	500	9	210	290	500	11	375	625	1000	20
100	198	302	500	17	243	257	500	23	441	559	1000	40

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes

MN = Micronuclei

Appendix 5. Individual incidences of micronuclei in the bone marrow of female mice in a study with Proxitan-0510 - 48 hour examination.

Treatment and Animal Number	Slide A				Slide B				Combined values					
	PCE	NCE	MN in NCE	Total PCE	PCE	NCE	MN in NCE	Total PCE	PCE	NCE	MN in NCE	Total PCE	MN in PCE	PCE
Control														
Saline														
101	260	240	0	500	0	216	0	500	0	544	0	1000	0	0
102	330	170	0	500	2	305	0	500	0	525	0	1000	2	2
103	351	149	0	500	0	232	0	500	0	619	0	1000	0	0
104	254	246	0	500	2	253	0	500	0	501	0	1000	2	2
105	233	167	1	500	0	143	0	500	0	590	1	1000	0	0
Proxitan-0510														
8 mg/kg														
106	329	171	0	500	0	228	0	500	1	601	0	1000	1	1
107	263	237	0	500	0	293	0	500	0	470	0	1000	0	0
108	302	198	0	500	2	247	0	500	1	555	0	1000	3	3
109	240	260	1	500	0	223	0	500	2	517	1	1000	2	2
110	258	242	0	500	1	196	1	500	1	562	1	1000	2	2
Proxitan-0510														
35 mg/kg														
111	285	215	0	500	0	220	0	500	3	565	0	1000	3	3
112	222	278	0	500	0	294	0	500	1	428	0	1000	1	1
113	257	243	0	500	0	229	0	500	2	528	0	1000	2	2
114	250	250	0	500	3	233	1	500	1	517	1	1000	4	4
115	236	264	1	500	1	264	0	500	0	472	1	1000	1	1
Proxitan-0510														
150 mg/kg														
116	333	167	1	500	0	269	0	500	0	564	1	1000	0	0
117	273	227	0	500	0	261	0	500	1	512	0	1000	1	1
118	197	303	0	500	1	305	0	500	1	392	0	1000	2	2
119	220	280	0	500	0	277	0	500	0	443	0	1000	0	0
120	256	244	0	500	1	268	0	500	3	488	0	1000	4	4
Cyclophosphamide														
100 mg/kg														
121	85	415	9	125	2	419	9	250	10	166	18	375	12	12
122	36	464	5	125	4	399	3	250	17	137	8	375	21	21
123	175	325	6	500	16	410	4	500	15	265	10	1000	31	31
124	33	467	0	125	1	448	3	250	3	85	3	375	4	4
125	120	380	4	500	6	302	4	500	8	318	8	1000	14	14

MN = Micronuclei

PCE = Polychromatic erythrocytes

NCE = Normochromatic erythrocytes

Appendix 6. Individual incidences of micronuclei in the bone marrow of female mice in a study with Proxitan-0510 - 72 hour examination.

Treatment and Animal Number	Slide A				Slide B				Combined values					
	PCE	NCE	MN in NCE	Total PCE	MN in NCE	MN in PCE	Total PCE	MN in NCE	MN in PCE	Total PCE	MN in NCE	MN in PCE	Total PCE	
<u>Control</u>														
<u>Saline</u>														
126	241	259	0	500	0	231	269	0	500	0	472	528	0	1000
127	236	264	0	500	2	201	299	1	500	1	437	563	1	1000
128	219	281	0	500	2	264	236	0	500	0	483	517	0	1000
129	211	289	0	500	0	264	236	0	500	0	475	525	0	1000
130	331	169	0	500	0	312	188	0	500	1	643	357	0	1000
<u>Proxitan-0510</u>														
<u>8 mg/kg</u>														
131	198	302	0	500	0	202	298	0	500	1	400	600	0	1000
132	243	257	0	500	1	266	234	0	500	2	509	491	0	1000
133	249	251	0	500	0	230	270	0	500	0	479	521	0	1000
134	190	310	0	500	1	274	226	0	500	0	464	536	0	1000
135	295	205	0	500	0	301	199	0	500	1	596	404	0	1000
<u>Proxitan-0510</u>														
<u>35 mg/kg</u>														
136	255	245	0	500	1	279	221	0	500	0	534	466	0	1000
137	300	200	0	500	0	287	213	0	500	0	587	413	0	1000
138	288	212	0	500	3	200	300	0	500	0	488	512	0	1000
139	235	265	1	500	1	242	258	0	500	0	477	523	1	1000
140	236	264	0	500	0	204	296	0	500	0	440	560	0	1000
<u>Proxitan-0510</u>														
<u>150 mg/kg</u>														
141	256	244	0	500	0	264	236	0	500	1	520	480	0	1000
142	315	185	0	500	1	270	230	0	500	0	585	415	0	1000
143	177	323	0	500	1	156	344	0	500	1	333	667	0	1000
144	182	318	0	500	1	235	265	0	500	0	417	583	0	1000
145	231	269	0	500	0	215	285	0	500	2	446	554	0	1000
<u>Cyclophosphamide</u>														
<u>100 mg/kg</u>														
146	64	436	3	125	5	57	443	6	125	1	121	879	9	250
147	27	473	1	125	0	27	473	2	250	1	54	946	3	375
148	61	439	0	500	2	77	423	0	125	1	138	862	0	625
149	45	455	0	250	0	69	431	1	250	1	114	886	1	500
150	50	450	5	250	1	34	466	1	250	1	84	916	6	500

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes

MN = Micronuclei

Appendix 7. Individual body weights of male mice in a micronucleus assay with Proxitane-0510.

Treatment and Animal number	Bodyweight (g) at time (days)					
	-7	-3	0	1	2	3
<u>Control</u>						
<u>Saline</u>						
1	26	27	27	28		
2	28	30	31	32		
3	28	29	28	29		
4	27	30	29	30		
5	27	28	28	29		
26	27	29	29		31	
27	28	30	30		31	
28	26	28	28		29	
29	26	30	30		32	
30	26	30	29		30	
51	28	30	30			31
52	26	30	30			32
53	29	31	30			32
54	29	33	32			35
55	30	32	32			34
<u>Proxitane-0510</u>						
<u>8 mg/kg</u>						
6	26	30	29	30		
7	24	26	26	29		
8	27	30	29	30		
9	26	21	26	29		
10	27	30	29	29		
31	28	29	30		31	
32	27	29	30		32	
33	30	33	32		33	
34	26	29	28		30	
35	28	32	31		33	
56	26	27	27			29
57	26	27	26			28
58	30	33	34			36
59	28	32	31			34
60	25	26	25			28

....continued

Appendix 7. (Continued).

Treatment and Animal number	Bodyweight (g) at time (days)					
	-7	-3	0	1	2	3
<u>Proxitane-0510</u>						
<u>35 mg/kg</u>						
11	27	30	30	30		
12	25	30	30	31		
13	27	30	30	30		
14	28	32	31	34		
15	26	29	28	29		
36	28	31	31		32	
37	26	30	29		30	
38	29	32	31		33	
39	25	26	26		27	
40	25	28	27		29	
61	27	30	30			32
62	25	28	28			30
63	27	30	29			32
64	28	30	30			33
65	26	27	27			29
<u>Proxitane-0510</u>						
<u>150 mg/kg</u>						
16	24	27	21	26		
17	26	30	31	30		
18	25	29	30	30		
19	22	20	27	29		
20	25	30	29	30		
41	25	28	28		30	
42	28	30	29		29	
43	27	29	28		29	
44	26	31	31		32	
45	27	30	31		33	
66	28	30	29			29
67	28	31	32			34
68	28	30	31			32
69	28	31	30			32
70	28	32	32			34

....continued

Appendix 7. (Continued).

Treatment and Animal number	Bodyweight (g) at time (days)					
	-7	-3	0	1	2	3
<u>Cyclophosphamide</u>						
<u>100 mg/kg</u>						
21	23	26	26	26		
22	27	32	32	32		
23	28	28	27	28		
24	26	30	30	30		
25	26	30	31	31		
46	27	30	30		31	
47	27	30	30		31	
48	30	32	32		33	
49	28	31	31		32	
50	25	29	28		30	
71	25	30	29			31
72	27	30	29			30
73	28	30	31			32
74	26	31	30			32
75	25	29	28			30

Appendix 8. Individual body weights of female mice in a micronucleus assay with Proxidane-0510.

Treatment and Animal number	Bodyweight (g) at time (days)					
	-7	-3	0	1	2	3
<u>Control</u>						
<u>Saline</u>						
76	24	26	21	26		
77	23	23	21	24		
78	25	26	23	25		
79	23	24	22	22		
80	23	23	23	25		
101	23	24	23		24	
102	24	26	26		26	
103	23	22	23		23	
104	21	22	21		22	
105	20	22	21		22	
126	22	23	23			25
127	23	24	24			26
128	22	22	22			22
129	22	23	22			23
130	23	23	22			22
<u>Proxidane-0510</u>						
<u>8 mg/kg</u>						
81	22	22	22	23		
82	22	23	22	26		
83	24	25	24	25		
84	24	25	24	26		
85	23	24	22	25		
106	22	22	21		22	
107	23	24	23		25	
108	22	22	21		23	
109	22	21	22		23	
110	23	24	21		23	
131	22	25	25			26
132	20	20	20			20
133	23	25	25			25
134	25	29	28			29
135	21	22	21			23

....continued

Appendix 8. (Continued).

Treatment and Animal number	Bodyweight (g) at time (days)					
	-7	-3	0	1	2	3
<u>Proxitane-0510</u>						
<u>35 mg/kg</u>						
86	22	24	23	24		
87	26	27	28	29		
88	19	22	22	23		
89	25	26	25	28		
90	23	23	18	23		
111	21	23	22		23	
112	25	27	25		28	
113	22	23	23		24	
114	24	25	23		26	
115	23	25	25		26	
136	23	23	21			25
137	24	26	24			27
138	20	20	20			21
139	21	22	21			23
140	23	24	23			24
<u>Proxitane-0510</u>						
<u>150 mg/kg</u>						
91	24	25	19	24		
92	22	21	21	22		
93	22	24	23	25		
94	22	23	21	22		
95	21	21	21	21		
116	21	21	21		22	
117	22	24	21		23	
118	21	24	23		22	
119	23	23	22		23	
120	24	25	24		26	
141	22	23	22			24
142	23	24	23			25
143	22	22	21			22
144	23	24	22			23
145	23	23	21			22

....continued

Appendix 8. (Continued).

Treatment and Animal number	Bodyweight (g) at time (days)					
	-7	-3	0	1	2	3
<u>Cyclophosphamide</u>						
<u>100 mg/kg</u>						
96	21	21	22	23		
97	21	22	21	23		
98	24	25	22	22		
99	22	24	24	25		
100	20	24	23	24		
121	25	27	26		28	
122	23	21	20		25	
123	22	22	22		22	
124	22	23	22		24	
125	23	24	22		24	
146	23	24	23			25
147	22	21	22			21
148	22	21	21			22
149	21	23	21			22
150	23	24	24			25



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

Paul J. Harding
Attorney
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Houston, Texas 77098

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

FEB 09 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information, and refer to the reverse side of this page for "EPA Information Requests".

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U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure

13267 A



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EP. INFORMATION REQUESTS

Document ID: 8EHQ-1194-13267

EPA requests:

1. No additional information at this time.
2. Additional information or clarification on

3. A full copy of the final report (including the actual experimental protocol, applicable results of gross or histopathologic examinations, data, results of any statistical analyses, etc.) from each study mentioned in your submission.
4. A description of all voluntary actions taken by your company in response to the findings indicated in your submission.
5. A complete copy of the current and/or revised Material Safety Data Sheets and labels for the following chemical(s) listed in your submission:

6.

Please direct questions regarding these requests to Mr. Terry O'Bryan (202-260-3483) or Mr. John Myers (202-260-3543) of the OPPT Risk Analysis Branch.

Triage of 8(e) Submissions

Date sent to triage: APR 06 1995

NON-CAP

CAP

Submission number: 13267A

TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX
STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

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entire document: <u>0</u> 1 2 pages <u> </u>	pages <u>1-3, tabs</u>
Notes: <u>2-sided</u>	
Contractor reviewer: <u>LPS</u>	Date: <u>1/30/95</u>

CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # BEHQ-1194-13067 SEQ. A
 TYPE: (INT) SUPP FLWP SUBMITTER NAME: Solvay Interox

INFORMATION REQUESTED: FLWP DATE
 0501 NO INFO REQUESTED
 0502 INFO REQUESTED (TECH)
 0503 INFO REQUESTED (VOL ACTIONS)
 0504 INFO REQUESTED (REPORTING RATIONALE)
 DISPOSITION:
(639) REFER TO CHEMICAL SCREENING
 0678 CAP NOTICE

VOLUNTARY ACTIONS:
 0401 NO ACTION REPORTED
 0402 STUDIES PLANNED/IN PROGRESS
 0403 MODIFICATION OF WORK PRACTICES
 0404 LABELS/MSDS CHANGES
 0405 PROCESS/HANDLING CHANGES
 0406 APP/USE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

SUB. DATE: 11/29/94 OTR DATE: 11/30/94 CSRAD DATE: 12/30/94
 CHEMICAL NAME: Proxitane - 0510 CASE: 79-21-0

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEMPHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUVA (IN VIVO)	01 02 04	0220 ECOAQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUR/REL FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQEST DELAY	01 02 04	0248 PRODUSE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PROD/COMP/CHEM ID	01 02 04	MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	METAB/PHARMACO (HUMAN)	01 02 04		

TRIAJE DATA: NON-CBI INVENTORY YES (circled) NO
 CAS SR: NO
 IS IN MRM: NO
 USE: disinfectant
sterilization
biocide
 SPECIES: In vitro LOW
Rat MED
mus HIGH
 ONGOING REVIEW: YES (DROP/REFER) NO (CONTINUE) NEVER
 TOXICOLOGICAL CONCERN: LOW MED HIGH

3)

8EHQ-1194-13267: Rank - medium.

Chemical: Proxitane-0150 (CAS# no given)
[primary component, peracetic acid (PAA), CAS# 790-21-0)].

The effects of Proxitane-0510 on the chromosomes on cultured human lymphocytes, BIBRA Toxicology International, Surrey, UK, signed by study director on 7 February 1994: Positive for chromosome mutations (aberrations) in human lymphocytes in vitro both without and with metabolic activation.

A micronucleus test with Proxitane 0510, BIBRA Toxicology International, Surrey, UK, dated 7 February 1994: Negative for chromosome mutations (micronuclei) in mice exposed by oral gavage in vivo.

An in vivo unscheduled DNA synthesis assay with Proxitane 0510, BIBRA Toxicology International, Surrey, UK, dated 17 August 1994: Does not induce DNA effects in the form of unscheduled DNA synthesis (UDS) in rat hepatocytes after in vivo treatment.