AD-A084 121 INVERESK RESEARCH INTERNATIONAL LTD EDINBURGH (SCOTLAND) F/G 6/5
MUTAGENICITY AND DNA REPAIR POTENTIAL OF 15 CHEMICALS,(U)
JAN 80 D B MCGREGOR DAMD17-78-C-8064

UNCLASSIFIED NL

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Final Report	2. GOVT ACCESSION NO.: AD-A084 121
3. RECIPIENT'S CATALOG NUMBER: (9)	
4. TITLE (and Subtitle): Mutagenicity and DNA Repair Potential of 15 Chemicals	5. TYPE OF REPORT & PERIOD COVERED: Final rept. 1 Oct 78 - 30 Nov 79
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Douglas B. McGregor	8. CONTRACT OR GRANT NUMBER(s): DAMD 17-78-C-8064
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Inveresk Research International Limited, Edinburgh EH21 7UB, Scotland	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research & Development Command, Fort Detrick, Frederick, MD 21701	12. REPORT DATE: January 1980
	13. NUMBER OF PAGES: 298 (12) 204
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): U.S. Army Medical Bioengineering Research & Development Laboratory, Fort Detrick, Frederick, MD 21701	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

DNA repair, mutation, mitotic recombination, E. coli, S. typhimurium. S. cerevisiae, Octahydro-1-acetyl-3,5,7-trinitro-S-tetramine (SEX), Hexahydro-1,3-dinitro-5-acetyl-S-triamine (TAX), Ethyl centralite, 2-Nitrodiphenylamine, Lead salicylate, Lead resorcylate, Diethyleneglycoldinitrate, Red phosphorus, Nitroguanidine, N-nitrosodiphenylamine, Diphenylamine, Zinc chloride,

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A research programme was established to construct a priority list of certain ordnance compounds for carcinogenic testing. As aids to this objective, a restricted battery of so-called short-term screening tests was used. In this initial part of the programme, 15 chemicals were tested.

Five strains of Salmonella typhimurium were used in mutagenicity tests according to the techniques established by Ames et al, (1975) Mutation Res., 31 347. Two strains of Escherichia coli were used in DNA repair tests on

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

19.
KEY WORDS (continued)

Tetryl, 1,3-Dinitrobenzene, 1,3,5-Trinitrobenzene.

20.
ABSTRACT (continued)

plates and, where necessary, in liquid medium. This latter technique was used only if the plate test for DNA repair failed to produce toxicity in either strain. A third test used was the mitotic recombinogenic activity assay in the yeast, Saccharomyces cerevisiae D_5.

Each test was conducted in vitro and in tissue (liver) mediated assays. Liver for these tests came from young, adult, male Fischer 344 rats following enzyme induction with aroclor 1254. Post-mitochondrial supernatant fluid fractions (S-9) were stored in liquid nitrogen (-192°C) for up to one month before they were discarded.

Failure to demonstrate potential for damaging genetic material resulted from tests with the following compounds:

Octahydro-1-acetyl-3,5,7-trinitro-S-tetramine (SEX), Hexahydro-1,3-dinitro-5-acetyl-S-triamine (TAX), Ethyl centralite, 2-Nitrodiphenylamine, Lead salicylate, Lead resorcylate, Diethyleneglycoldinitrate, Red phosphorus, Nitroguanidine, N-nitrosodiphenylamine, Diphenylamine

While the bacterial tests with a zinc chloride sample were negative, some reservations were expressed concerning the recombinogenic activity of this compound in the absence of S-9 mix. The slight indication of recombinogenic activity in this test was not confirmed in the test performed with S-9 mix.

Three compounds were demonstrated as genetically active in these experiments:

Tetryl, 1,3-Dinitrobenzene, 1,3,5-Trinitrobenzene

Genetic activity of tetryl was demonstrated with S. typhimurium TA 1537, TA 1538, TA 98 and TA 100. The responses of these strains were particularly strong in the absence of S-9 mix. Tetryl also induced increases in recombinant numbers and frequencies in the S. cerevisiae test without S-9 mix. Increases in recombinant frequency were seen in 3 experiments while an increase in recombinant number was seen in one test. This activity was not observed in the presence of S-9 mix.

1,3-Dinitrobenzene was demonstrated as a mutagen with S. typhimurium TA 1538, TA 98 and TA 100. Slight activity was also seen with strain TA 1537. S-9 mix reduced the magnitude of the responses.

1,3,5-Trinitrobenzene was demonstrated as a mutagen with S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Again, S-9 mix reduced the magnitude of the responses.

The analytical quality of the 3 substances giving positive responses was investigated and the results are included in this report.

AD _____

MUTAGENICITY AND DNA REPAIR POTENTIAL OF 15 CHEMICALS

FINAL REPORT

By

Douglas B. McGregor

January 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

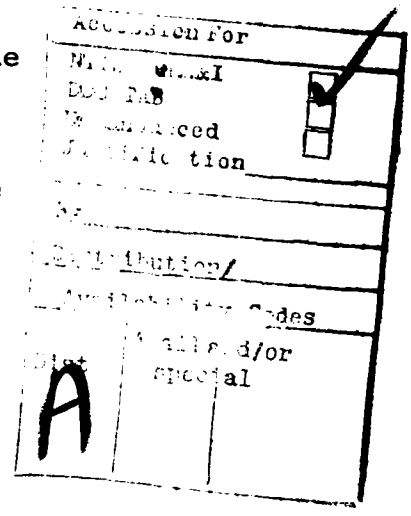
Contract No. DAMD 17-78-C-8064

Inveresk Research International Limited
Edinburgh EH21 7UB, Scotland

Contracting Officer's Technical Representative
Dr Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

CONTENTS

CONTENTS (continued)



LOCATION OF EXPERIMENTAL PROGRAMME

The experiments described in this report were performed in their entirety at the Inveresk Gate laboratories of Inveresk Research International Limited, Edinburgh, Scotland between October 1978 and November 1979.

The original data will be held in the Quality Assurance Unit Archives of Inveresk Research International Limited for 5 years.

PERSONNEL INVOLVEMENT

Principal Investigator

Douglas B. McGregor, B.Sc., Ph.D.

Scientific and Technical Staff

Geoff A. Byrne, C.Chem., F.R.I.C., Res.Dip.RIC
Christopher Corden, B.Sc.
John N. Done, B.Sc., Ph.D.
John D. Gilbert, B.Sc., Ph.D., C.Chem., M.R.I.C.
Anne Gilroy
Jennifer Harvey, B.Sc.
Rowan Hastwell, B.Sc.
Margaret S. Henderson, B.Sc., Ph.D.
Colin Riach, B.Sc.
Anne Scott, B.Sc.
Philip Teale, B.Sc.

EXECUTIVE SUMMARY

A research programme was established to construct a priority list of certain ordnance compounds for carcinogenic testing. As aids to this objective, a restricted battery of so-called short-term screening tests was used. In this initial part of the programme, 15 chemicals were tested.

Five strains of Salmonella typhimurium were used in mutagenicity tests according to the techniques established by Ames et al, (1975) Mutation Res., 31 347. Two strains of Escherichia coli were used in DNA repair tests on plates and, where necessary, in liquid medium. This latter technique was used only if the plate test for DNA repair failed to produce toxicity in either strain. A third test used was the mitotic recombinogenic activity assay in the yeast, Saccharomyces cerevisiae D_5.

Each test was conducted in vitro and in tissue (liver) mediated assays. Liver for these tests came from young, adult, male Fischer 344 rats following enzyme induction with aroclor 1254. Post-mitochondrial supernatant fluid fractions (S-9) were stored in liquid nitrogen (-192°C) for up to one month before they were discarded.

Failure to demonstrate potential for damaging genetic material resulted from tests with the following compounds:

Octahydro-1-acetyl-3,5,7-trinitro-S-tetramine (SEX)
Hexahydro-1,3-dinitro-5-acetyl-S-triamine (TAX)
Ethyl centralite
2-Nitrodiphenylamine
Lead salicylate
Lead resorcylate
Diethyleneglycoldinitrate
Red phosphorus
Nitroguanidine
N-nitrosodiphenylamine
Diphenylamine

While the bacterial tests with a zinc chloride sample were negative, some reservations were expressed concerning the recombinogenic activity of this compound in the absence of S-9 mix. The slight indication of recombinogenic activity in this test was not confirmed in the test performed with S-9 mix.

Three compounds were demonstrated as genetically active in these experiments:

Tetryl
1,3-Dinitrobenzene
1,3,5-Trinitrobenzene

Genetic activity of tetryl was demonstrated with S. typhimurium TA 1537, TA 1538, TA 98 and TA 100. The responses of these strains were particularly strong in the absence of S-9 mix. Tetryl also induced increases in recombinant numbers and frequencies in the S. cerevisiae test without S-9 mix. Increases in recombinant frequency were seen in 3 experiments while an increase in recombinant number was seen in one test. This activity was not observed in the presence of S-9 mix.

1,3-Dinitrobenzene was demonstrated as a mutagen with S. typhimurium TA 1538, TA 98 and TA 100. Slight activity was also seen with strain TA 1537. S-9 mix reduced the magnitude of the responses.

1,3,5-Trinitrobenzene was demonstrated as a mutagen with S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Again, S-9 mix reduced the magnitude of the responses.

The analytical quality of the 3 substances giving positive responses was investigated and the results are included in this report.

INTRODUCTION

In its efforts to safe-guard the health of its work force and the American people in general, the U.S. Army wished to integrate within its toxicology programme a scheme for screening for potential carcinogenic activity. The screen conducted was based on mutagenicity testing and the assessment of DNA damage in micro-organisms.

Mutagenicity is a young branch of science in which the participants are, on the whole, anxious to arrive at a balanced view of the importance of environmental mutagens to individuals and to human populations. Therefore, when faced with the large number of different test systems, concerned people have attempted to classify these systems in order to give them graded importance. Generally, 3 grades, levels or tiers have been advocated. The first tier is an initial screening effort in which it is hoped that all mutagens would be found. The second tier is intended to confirm the results of the first tier and demonstrate activity in complex animals, especially mammals. In tier III, the attempt is made to estimate quantitatively the mutagenic risk to man.

The work carried out in this programme was tier I testing of 15 compounds.

Tier I tests in any tier system widely discussed are wholly sub-mammalian. They may include tests with Drosophila, DNA repair assays, gene conversion or recombination in yeasts, mutation induction in cultured mammalian cells, chromosomal aberration induction in vitro or malignant cell transformation in vitro. But the best validated genetic test available at present is the one involving the mutation of bacteria in the presence of a microsomal fraction of mammalian liver. Validation does not mean, however, that the test is relevant for predicting mutagenicity in man: there is not the information available to allow that sort of statement to be made. No, validation only means, in this instance, that the results of the microbial mutation test show a promising parallel with the results of mammalian bioassays for carcinogenic activity. These bacterial tests form part of the work programme proposed and results from such tests were to be refuted or substantiated by tests for recombinogenic activity in yeasts.

OBJECTIVES AND SCOPE

The objective of the research was to subject a number of compounds used by the U.S. Army in ordnance to a screening programme for potential carcinogens.

Aquatic studies are being conducted in-house by the U.S. Army and environmental impact is also being evaluated under contract.

Fifteen compounds were included in the programme and, in view of the protracted character of mammalian bioassays for carcinogens, a rational framework had to be established to list these compounds in order of priority for more extensive toxicological evaluation. In order to meet this need, each compound was tested for:

(a) induction of reversions in Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100;

(b) induction of DNA damage in Escherichia coli W3110 polA$^+$ and p3478 polA$^-$ strains;

(c) induction of mitotic recombination in Saccharomyces cerevisiae D_5.

These tests cover a variety of potential damage. Should a substance come through this test programme without having shown activity in any of these tests, then it should be placed very low on a table of priorities for further testing for long term effects. On the other hand, should a substance be active in some or all of these tests then it should be examined carefully in order to properly evaluate its toxic potential.

BACKGROUND

An important goal in toxicology is the prevention of cancer in man by identifying those substances with carcinogenic potential which may affect humans. As stated by a past Director of the National Cancer Programme (1), "At an accelerating rate, we are adding thousands of products of our modern technology to the environment in which we live and work. We have come to accept as a scientific judgment the view that our present environment is dangerous and that it is responsible for a vast majority of cancers ..... The need is thus greater now than ever to define carcinogenic hazards and uncover the means for cancer prevention."

The U.S. Army is dealing with a substantial list of compounds which are used in ordnance and are now to be evaluated for their toxicological impact, both on the environment generally and to man, as long term hazards, in particular. It is important when dealing with a substantial list that a rational approach be adopted in the priority choice of chemicals to proceed to bioassay.

Bases for a rational choice would seem to reside in 2 areas of scientific research. These areas are (i) mammalian cell transformation and (ii) mutagenicity and DNA damage in mammalian cells or micro-organisms. It has been repeatedly shown that cells transformed by physical or chemical agents do produce tumours when injected into mammalian hosts (2). This approach to carcinogenicity testing has been considerably improved by the use of host mediated assays (3). Nevertheless, these transformation tests must be considered as second tier tests and the 'better' ones do take an appreciable time to conduct. The use of DNA repair tests and mutagenicity tests as indicators of carcinogenic potential of chemicals is founded, in the minds of many carcinogenesis researchers, on the belief that the 'permanent' change manifest in neoplastic cells is due to the expression of genetic alterations.

The somatic cell mutation theory of the origins of cancers has been an attractive theory for many years, but only in recent years have circumstantial data been built up to support the theory. At the 1974 Honolulu workshop (4) it became apparent that the best correlation or parallel was between carcinogenesis and microbial systems for mutation induction or DNA repair in Salmonella typhimurium, Escherichia coli and Bacillus subtilis in combination with in vitro metabolic activation media. If the data from various systems were pooled then the parallel was even better.

The findings of the Honolulu meeting were consolidated by those of the Seattle meeting in 1975 (5). Here, McCann reported that about 80% of chemical carcinogens are mutagens and about 10% non-carcinogens are mutagens. It had, by that time, become clear that the in vitro liver homogenate systems are superior to the host mediated assay. Also, DNA repair tests with E. coli p3478 and W3110 strains are more selective than assays using B. subtilis H17 and M45 or S. typhimurium TA 1789 and TA 1538 strains. Pienta, at this same meeting, reported that 58/65 (i.e. 89%) known carcinogens induced transformation of hamster embryo cells in vitro.

The message from these conferences was clear: that carcinogenicity could be predicted using short term tests and that a battery of tests should be used to provide confirmation of positive test data and to reduce the possibility of false negative tests. The same conclusion is reached upon examination of Imperial Chemical Industries Limited, Central Toxicology Laboratory's programme of testing 120 coded chemicals. In this programme an E. coli repair test was not used, but there was an in vitro cell transformation assay using BHK 21 cl.13 cells and a S. typhimurium plate incorporation test. With either system there was about 90% correct prediction of carcinogenic activity. If the results are combined, however, the prediction is 99% correct, only diethylstilbestrol being wrongly called non-carcinogenic. The BHK 21 cl.13 cell transformation assay system has, however, been heavily criticised on the grounds that the cells are already transformed and the degree of cloning in soft agar (the end point measured) is already high prior to treatment with a carcinogen.

A positive indication of activity in a bacterial test is clearly important, but the weight of such evidence is heavily modified by results from tests using eukaryotic cells.

The other test system which has been included in this work programme, is the yeast recombination test. It lacks the very high sensitivity observed with bacterial tests and only small effects are seen even with potent mutagens, such as several nitrosamines.

The results of recombinogenic tests can be important, however, in grading the importance of a group of chemicals, all of which give positive results in bacterial mutation or DNA repair tests.

MATERIALS AND METHODS

Chemicals

Fifteen chemicals were tested in assays for genetic damage. These were:

1. Octahydro-1-acetyl-3,5,7-trinitro-S-tetramine (SEX). Supplied by U.S. Army (Holston Defense Corpn). 2.0 g received on 1 June 1979.

2. Hexahydro-1,3-dinitro-5-acetyl-S-triamine (TAX). Supplied by U.S. Army (Holston Defense Corpn). 2.0 g received on 1 June, 1979.

3. Ethyl centralite. Supplied by Ministry of Defence, Waltham Abbey. Coded 3/77 Ref. QC/976/77. 5.0 g received on 14 November 1978.

4. 2-Nitrodiphenylamine. Supplied by Ministry of Defence, Waltham Abbey. Coded 4537/290. 5.0 g received on 14 November 1978.

5. Lead salicylate. Supplied by Ministry of Defence, Waltham Abbey. Coded LN132. 5.0 g received on 14 November 1978.

6. Lead resorcylate. Supplied by Ministry of Defence, Waltham Abbey. Ex. Hopkins and Williams SO/NO14768. 5.0 g received on 14 November 1978.

7. Diethyleneglycoldinitrate. Supplied by Ministry of Defence, Waltham Abbey. Ex. PR stock. 5.0 g received on 14 November 1978.

8. Tetryl. Supplied by Ministry of Defence, Waltham Abbey. Ex. Wartime stock. 5.0 g received on 14 November 1978.

9a. Red Phosphorus. Supplied by Ministry of Defence, Waltham Abbey. No identification. Received on 9 October 1979 (not used).

9b. Red Phosphorus. Supplied by B.D.H. Limited, Poole, Dorset. Batch no. 6111460. 100 g received on 3 November 1978.

10a. Nitroguanidine. Supplied by Ministry of Defence, Waltham Abbey. Coded 7227. 5.0 g received on 14 November 1978.

10b. Nitroguanidine with 20% water. Supplied by Fluka AG, Switzerland. Batch no. 755377. 100 g received (not used).

11. N-Nitrosodiphenylamine. Supplied by Ministry of Defence, Waltham Abbey. Coded PH2/76/22A.

12. Diphenylamine. Supplied by Ministry of Defence, Waltham Abbey. Coded 58711 W3 1975.

13. 1,3-Dinitrobenzene (organic analytical standard grade). Supplied by B.D.H. Limited, Poole, Dorset. Batch no. 6105910. 25 g received on 3 November 1978.

14. 1,3,5-Trinitrobenzene. Supplied by Ministry of Defence, Waltham Abbey. Coded NP/72/78. 5.0 g received on 14 November 1978.

15. Zinc chloride. Supplied by Ministry of Defence, Waltham Abbey. Coded 222314301/1. 5.0 g received on 14 November 1978.

All of these chemicals were kept at ambient room temperature in a locked steel cupboard of design approved by H.M. Explosives Inspectorate, Health and Safety Executive.

Positive control substances used were:

1. 2-Aminoanthracene (S. typhimurium + S-9 mix), Aldrich Chemical Company, Gillingham, Dorset.

2. 2-Nitrofluorene (S. typhimurium, TA 1538, TA 98), IIT Research Institute, Chicago, Illinois.

3. 9-Aminoacridine (S. typhimurium, TA 1537) B.D.H. Limited, Poole, Dorset.

4. Sodium azide (S. typhimurium, TA 1535, TA 100), B.D.H. Limited, Poole, Dorset.

5. Ethyl methanesulphonate (E. coli, yeast), Koch Light Limited, Colnbrook, Bucks.

6. Chloramphenicol (E. coli), Sigma (London) Chemical Company.

7. 2-Aminofluorene (E. coli), Koch Light Limited, Colnbrook, Bucks.

8. Cyclophosphamide (yeast), Koch Light Limited, Colnbrook, Bucks.

9. N-Nitrosodimethylamine (yeast), Aldrich Chemical Company, Gillingham, Dorset.

Aroclor 1254, used to induce the liver enzymes, was obtained from IIT Research Institute, Chicago, Illinois.

Animals

Male Fischer 344 rats (Bantin and Kingman Limited, Hull, England)weighing 250-300 g were used. They were obtained in batches of 10 at monthly intervals as required by the project. They were injected intraperitoneally (i.p.) with aroclor 1254 (diluted in corn oil to a concentration of 200 $mg.ml^{-1}$) at a dosage of 500 $mg.kg^{-1}$ 5 days before they were killed. The animals were allowed drinking water continuously, but food was withdrawn 16 h before they were killed by dislocation of their necks.

Micro-organisms

Salmonella typhimurium

Five strains of S. typhimurium were used:

S. typhimurium	TA 1535
" "	TA 100
" "	TA 1537
" "	TA 1538
" "	TA 98

All these strains contain mutations in the histidine operon, thereby imposing a requirement for histidine in the growth medium. Three mutations in the histidine operon are involved.

his G 46 in TA 1535 and TA 100
his C 3076 in TA 1537
his D 3052 in TA 1538 and TA 98

his G 46 is a mis-sense mutation which is reverted to prototrophy by a variety of mutagens that cause base-pair substitutions.

his C 3076 contains a frameshift which appears to have added a -G- base-pair resulting in -GGGG-. This mutation is reverted by 9-aminoacridine, ICR-191 and epoxides of polycyclic hydrocarbons.

his D 3052 also contains a frameshift mutation with the sequence -CGCGCG- which is reverted with the deletion of 2 base-pairs, -CG-. It is readily reverted by aromatic amines and derivatives.

All 5 strains contain the deep rough (rfa) mutation, which deletes the polysaccharide side chain of the lipopolysaccharide coat of the bacterial cell surface. This deletion increases cell permeability to more hydrophobic substances and, furthermore, greatly decreases the pathogenicity of these organisms.

The second deletion, through uvrB, renders the organisms incapable of DNA excision repair and thus more susceptible to mutagenicity. These 2 deletions include the nitrate reductase (chl) and biotin (bio) genes also.

Differences between TA 1535 and TA 1538, on one hand, and the corresponding TA 100 and TA 98 strains on the other hand, are due to a plasmid the latter pair contain. A plasmid, R-Utrecht, was originally shown to increase the sensitivity of the his G 46 mutation in S. typhimurium to methyl methanesulphonate and trimethyl phosphate. The particular R-factor in TA 100 and TA 98 carries resistance to ampicillin. It is not yet clear why the presence of this particular R-factor should increase the sensitivity of strains TA 1535 and TA 1538 to the mutagenicity of certain chemicals. The involvement of an error-prone repair mechanism has been postulated.

Escherichia coli

E. coli W3110/polA$^+$ and p3478/polA$^-$ strains were used in tests for lethality due to damage to DNA. The polA$^-$ strain contains less than 1% of the normal level of extractable DNA polymerase, but multiplies normally. It contains an amber, non-sense, mutation and shows an increased sensitivity to ultraviolet light and to ethyl methanesulphonate (6). The polA$^+$ strain contains the normal quantity of extractable DNA polymerase.

Saccharomyces cerevisiae D_5

Strain D_5 was used to detect the induction of mitotic crossing over, a reciprocal mitotic recombination that results in the change in the sequence of different genes along one chromosome region. Strain D_5 is diploid and recombinational events are indicated by the phenotypic expression at the gene locus ade 2. Defective mutants of ade 2 require adenine for growth and in its absence accumulate a red pigment.

D_5 ade 2-40: this mutant has an absolute growth requirement for adenine and on low-adenine media gives deep red colonies.

D_5 ade 2-119: this is a 'leaky' mutant and its growth rate is only reduced in the absence of adenine. On a low-adenine medium, the colonies are pink instead of red.

In diploid cells carrying a heteroallelic combination, the 2 alleles complement each other and the colonies produced are white and have no adenine requirement.

If mitotic crossing-over occurs between the centromere and the ade 2 locus this leads to segregation of the daughter nuclei in 50% of them. As a result, one nucleus is homoallelic ade 2-119 and colonies are formed that have a red and pink sector: twin-spotted colonies. The presence of twin red-pink spots in strain D_5 is generally regarded as positive proof of reciprocal mitotic recombination and D_5 is recognised as the most reliable strain to directly measure the induction of true mitotic recombination.

Mutagenic treatment of D_5 can result in all red or all pink colonies or a variety of sectored colonies: red-pink, red-pink-white, white-pink, red-white. The first 2 are probably due to the induction by treatment of mitotic gene conversion (the unilateral transfer of small lengths of DNA, up to about 100 nucleotides long, between homologous regions of chromatids of homologous chromosomes), point mutations, chromosomal deletions and aneuploidy. The strain is, therefore, a multipurpose strain, but only mitotic crossing-over can be unequivocally demonstrated without further genetic analysis.

At high survival levels the frequency of mitotic crossing-over shows a consistent correlation with cell survival irrespective of the inducing agent. It has been suggested that mitotic crossing-over is produced in cells carrying damage not repairable by either excision or post-replication repair, but by the repair of double strand breaks and, therefore, reconstruction of the broken chromosome fragments.

Preparation of the 9,000 g supernatant fluid from livers

Freshly killed animals were thoroughly swabbed with 70% ethanol, the abdomens opened and livers removed, taking special care not to cut into the gastro-intestinal tract. The livers were collected in tared beakers containing ice-cold homogenisation medium. The medium used was 0.15 M-KCl.

The beakers were weighed and the collected livers transferred to the homogenisation vessel. A volume of ice-cold 0.15 M-KCl equivalent to 3 times the weight of the liver was added to the vessel and the livers chopped using long-handled scissors. The chopped livers were homogenised by 8 strokes of a glass tube vessel while the Teflon pestle (radial clearance 0.14-0.15 mm) was rotating at about 1,200 r.p.m. The homogenate was transferred to sterile polypropylene centrifuge tubes and spun to give 9,000 g for 10 min at 0° to $+2^{\circ}$C. The supernatant fluid was decanted and retained, leaving behind a thick pellet of (mainly) whole cells, nuclei and mitochondria. Post-mitochondrial supernatant fluids were prepared in sufficient quantity for up to one month of the experiment and stored in liquid

nitrogen. Liver preparations stored in this way are stable for very long periods, but in the programme they were not used for longer than 4 weeks.

Testing with Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98

Samples of each strain were grown up by culturing for 16 h at 37°C in nutrient broth (8 g Bacto-Difco nutrient broth, 5 g $NaCl.l^{-1}$).

Ice-cold 0.05 M-phosphate buffer, pH 7.4, was added to pre-weighed NADP and glucose-6-phosphate, etc., as follows:

NADP-di-Na-salt	4 mM (= 3.366 $mg.ml^{-1}$)
Glucose-6-phosphate-di-Na-salt	5 mM (= 1.521 $mg.ml^{-1}$)
$MgCl_2.6H_2O$	8 mM (= 1.626 $mg.ml^{-1}$)
KCl	33 mM (= 2.460 $mg.ml^{-1}$)

This solution was immediately filter-sterilised by passage through a 0.45 μm Millipore filter and mixed with the liver 9,000 g supernatant fluid in the following proportion:

co-factor solution	9 parts
liver preparation	1 part

This combination of co-factors and liver preparation was called the "S-9 mix".

Diluted agar (0.6% Bacto-Difco agar, 0.5% NaCl) was autoclaved and just before use, 10 ml of sterile 0.5 mM-L-histidine HCl 0.5 mM-biotin solution was added to each 100 ml of soft agar and thoroughly mixed. This molten agar was dispensed in 2 ml volumes during the test. To this was added in order:

0.5 ml	"S-9 mix" or 0.05 M-phosphate buffer, pH 7.4
0.1 ml	bacteria (ca 2 x 10^9 $cells.ml^{-1}$)
0.2 ml	solvent or test solution

The tube contents (which were continually cooling) were mixed then poured onto minimal medium plates. These plates contained 20 ml of 1.5% Bacto-Difco agar in Vogel-Bonner Medium E (7) with 2% glucose. For each cell strain and chemical concentration, 3 replicate plates were prepared. When the soft agar had set, the plates were inverted and incubated at 37°C for 2 days and colonies counted using a New Brunswick Inc. (New Brunswick, N.J.) Biotran II automated counter set for maximum sensitivity (colonies of 0.1 mm or more in diameter counted). The plates were also examined for precipitates and, microscopically, for micro-colony growth.

The exposure range used was determined for each chemical in toxicity tests with strain TA 98.

Testing with E. coli W3110/polA$^+$ and p3478/polA$^-$

Samples of each strain were grown up by culturing for 16 h at 37°C in nutrient broth (8 g Bacto-Difco nutrient broth 5 g NaCl.l^{-1}). Such cultures may be kept for up to one week at +4°C, but they were normally used within 24 h.

Ice-cold 0.05 M-phosphate buffer, pH 7.4, was added to pre-weighed NADP and glucose-6-phosphate, etc., as follows, to give a final concentration in the 'S-9 mix' of:

NADP-di-Na-salt	4 mM (= 3.366 mg.ml^{-1})
Glucose-6-phosphate-di-Na-salt	5 mM (= 1.521 mg.ml^{-1})
$MgCl_2.6H_2O$	8 mM (= 1.626 mg.ml^{-1})
KCl	33 mM (= 2.460 mg.ml^{-1})

This solution was immediately filter sterilised by passage through 0.45 μm Millipore filter and mixed with the liver 9,000 g supernatant fluid in the following proportion:

co-factor solution	9 parts
liver preparation	1 part

The basis for the remainder of the method was the paper by Slater et al (8), but the procedures originally described by this group have been modified several times (e.g., Longnecker et al (9) and are modified again here.

Plate Test

A problem envisaged was in the nature of the spot test with E. coli W3110/polA$^+$ and p3478/polA$^-$. Certain essential components of the reaction mixture are water soluble (e.g., NADP, glucose-6-phosphate) whereas other components are not readily diffusible (e.g., microsomes). Hence, there is the opportunity for these components to separate. Similarly, a water soluble test substance is likely to diffuse from the central well. Where such a substance is an active metabolite a clear effect may be seen in this test, whereas, if the substance is water soluble prior to activation, its DNA damaging potential will appear to be low. Less hydrophilic metabolites may tend to dissolve in the lipoproteins of the non-diffusible liver cell organelles. In this latter case an active metabolite may show little effect in the repair synthesis test.

The following modifications of the Slater et al (7) method were adopted to overcome these theoretical limitations of the DNA repair test.

Plates were poured of Medium HA (10) containing 1.5% Bacto-Difco agar and supplemented with 5 μg thymine.ml^{-1} (8). A soft agar overlay was made using the same Medium HA and thymine supplement, but also containing 0.6% Bacto-Difco agar. To 2 ml of this soft agar, which was to be poured on each plate there was added the following:

0.1 ml *E. coli* W3110/polA^{+}
or
0.2 ml *E. coli* p3478/polA^{-}
and
0.5 ml S-9 mix
or
0.5 ml 0.05 M-phosphate buffer, pH 7.4

When the soft agar had set, a 13 mm diameter hole was cut into the middle of each plate. The bottom of each well was lined with 0.1 ml agar. When this had set, 0.1 ml test substance was added and the plates incubated for 16 h. At the end of this period, the diameter of the growth inhibition zone was measured and recorded. The test substance was dissolved in a suitable solvent and tested in triplicate.

Suspension Test

0.1 ml of log phase *E. coli* W3110 or p3478 diluted to 10^4 viable cells per ml were added to a 10 ml, γ-irradiated, plastic tube; this was followed by 0.5 ml S-9 mix or 0.05 M-phosphate buffer and 0.05 ml vehicle or test substance solution. The tube contents were then mixed and incubated in a gyrotary water bath for 1.5 h at 37°C. At the end of this period, 2.0 ml 0.6% agar containing medium HA and 5 μg thymine.ml^{-1} were added to each tube and the contents mixed. The tube contents were then poured onto a medium HA, 1.5% agar plate supplemented with 5 μg thymine.ml^{-1} and incubated at 37°C for 24 h.

Colonies were counted and survival compared in vehicle control and test agent plates. The relative toxicity of the test substance for the polA^{+} and polA^{-} strains was evaluated.

Testing with *S. cerevisiae* D_5

The yeast cells were grown up in a complete medium - YEP glucose (1% yeast extract, 2% peptone, 2% glucose) at 28°C. Approximately 200 cells were plated on to each of 5 plates of a synthetic complete medium (based on Difco yeast nitrogen base and supplemented with various amino acide and 2% glucose and containing 1.5% agar) with adenine at 5 mg.l^{-1} and incubated at 28°C for 4 days.

Synthetic Complete Medium

Difco yeast nitrogen base without animo acids	6.7 $g.l^{-1}$
Adenine sulphate	5 $mg.l^{-1}$
L-Arginine-HCl	10 $mg.l^{-1}$
L-Histidine-HCl	10 $mg.l^{-1}$
L-Isoleucine	60 $mg.l^{-1}$
L-Leucine	60 $mg.l^{-1}$
L-Lysine-HCl	10 $mg.l^{-1}$
L-Methionine	10 $mg.l^{-1}$
L-Tryptophan	10 $mg.l^{-1}$
L-Valine	30 $mg.l^{-1}$
Uracil	10 $mg.l^{-1}$

During the course of the programme, the recombinogenic activity test was modified substantially when S-9 mix was used. Hence, 2 methods are described. The toxicity test used with method 2 also changed; this modification also is described below.

Method 1

Cells carrying a heteroallelic combination, therefore producing all-white colonies since they have no adenine requirement, were picked off and used to form a stock suspension in 0.05 M-phosphate buffer, pH 7.4, of approximately 2×10^{7} cells.ml^{-1}.

The test compound was dissolved in a suitable vehicle miscible with water until a saturated solution was obtained. This was used as the highest exposure level in a toxicity test in which 5 plates per concentration were used. These were incubated as required in the main test (see below) and only total colonies counted.

Seven doses were chosen on the basis of toxicity test results.

Ice-cold 0.05 M-phosphate buffer, pH 7.4, was added to pre-weighed NADP and glucose-6-phosphate, etc., as follows to give in each ml of the 'S-9 mix':

NADP-di-Na-salt	12 mM (10.098 $mg.ml^{-1}$)
Glucose-6-phosphate-di-Na-salt	98 mM (29.812 $mg.ml^{-1}$)
$MgCl_2.6H_2O$	8 mM (1.626 $mg.ml^{-1}$)
KCl	33 mM (2.460 $mg.ml^{-1}$)

This solution was immediately filter sterilised by passage through a 0.45 µm Millipore filter and mixed with the liver 9,000 g supernatant fluid in the following proportion:

co-factor solution 9 parts
liver preparation 1 part

This combination of co-factors and liver preparation was called the 'S-9 mix'.

To a series of tubes in a water bath were added the following components:

0.5 ml samples of test compound solution (allowed to equilibrate to 28°C in water bath)

1.0 ml samples of the 'S-9 mix' or 0.05 M-phosphate buffer, pH 7.4

0.5 ml samples of the stock suspension of yeast cells.

The tube contents were mixed for the appropriate time (up to 2 h) at 28°C.

The treatment was stopped by diluting 1:100 with ice-cold water. The mixtures were then diluted a further 1:4 and 0.1 ml spread on to a synthetic complete medium containing 5 $mg.ml^{-1}$ of adenine sulphate and incubated at 28°C. In the main experiment 100 plates per concentration were used.

Pigment development is variable, but by following incubation at 28°C for 6 days with incubation at 4°C for 2 days pigment development was enhanced.

The aberrant colonies were listed in the following categories:

(i)	pink-red
(ii)	pink-red-white
(iii)	pink
(iv)	red
(v)	white-pink
(vi)	white-red
(vii)	hairline sectors - very tiny red or pink sectors where red and pink cannot be distinguished.

Method 2

Four or 5 all-white colonies from adenine-supplemented plates were picked off and grown up in 50 ml complete medium (1% yeast extract, 2% peptone, 2% glucose, pH 7.0) in a 100 ml conical flask for 20 h at 28°C on an orbital shaker at 50 r.p.m.

The cells in these log phase cultures were counted, then spun down just before they were required and resuspended in 0.25 x concentration complete medium (CM) and diluted with the same 0.25 CM to give approximately 1 x 10^7 cells. ml^{-1}. When dispensing the cells, the flask contents were vortex mixed, then kept upon a magnetic stirrer while pipetting was in progress.

Ice-cold 0.05 M-phosphate buffer, pH 7.4, was added to pre-weighed NADP and glucose-6-phosphate, etc., as follows to give in each ml of the "S-9 mix":

NADP-di-Na-salt	10.00 $mg.ml^{-1}$
Glucose-6-phosphate-di-Na-salt	27.65 $mg.ml^{-1}$
$MgCl_2.6H_2O$	1.62 $mg.ml^{-1}$
KCl	2.46 $mg.ml^{-1}$

This solution was filter sterilised by passage through 0.45 µm Millipore and mixed with S-9:

co-factor solution	9 parts
liver preparation	1 part

The reaction mixture was constituted as follows:

0.1 ml	S-9 mix
0.1 ml	test compound
1.8 ml	cell suspension in 0.25 CM

This mixture was incubated for 18 h at 28°C on an orbital shaker. The incubation bottles were capped McCartney bottles.

It should be noted that, during the incubation period, there was a 10-fold increase in cell number.

A preliminary toxicity test was run in which the conditions described above were used, but the number of plates was restricted to 5 per exposure level.

Furthermore, attempts were made to equalise the number of colonies likely to appear on the plates after incubation. This was done by making cell counts with a haemacytometer and modifying the dilutions at each exposure level accordingly. Success was not guaranteed with this technique, but the variation between exposure levels in colony numbers was reduced.

Plating and aberrant colony scoring methods were the same in method 2 as in method 1.

RESULTS

OCTAHYDRO-1-ACETYL-3,5,7-TRINITRO-S-TETRAMINE (SEX)

E. coli DNA Repair Tests on Plates (Table 1)

SEX was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no sign of toxicity in either strain in either the absence or presence of S-9 mix.

E. coli DNA Repair Tests in Suspension (Tables 2 and 3)

The highest exposure level used was 7.7 mg SEX.ml^{-1} incubation mixture. At this concentration precipitation occurred, but there was no toxicity in either strain in either the absence of presence of S-9 mix.

S. typhimurium Mutation Tests (Tables 4-6)

Preliminary toxicity tests with strain TA 98 indicated that SEX was not toxic to the bacteria, even at the very high exposure level of 10 mg per plate (Table 4). At this exposure level, SEX precipitated.

The mutagenicity tests were performed using S. typhimurium strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 5 and 6). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals between 10 µg and 10 mg per plate.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 7-10)

Toxicity tests were conducted in the absence and presence of S-9 mix using exposure levels up to 10 mg.ml^{-1} incubation mixture for 2 h. There were no indications of toxicity under any of these conditions (Table 7).

Tests for recombinogenic activity were performed using the method 1 incubation conditions in the absence of S-9 mix only. There was no indication that SEX possessed recombinogenic potential. The positive control substance, EMS, on the other hand induced high levels of recombination at an exposure level of 10 mg.ml^{-1} incubation mixture for 2 h (Table 9).

Using method 2 incubation conditions in the presence of S-9 mix, again there was no evidence of recombinogenic potential, there being no increase in the frequency or

number of recombinants recovered over the exposure range 78 $\mu g.ml^{-1}$ to 5 $mg.ml^{-1}$ (Table 10). This last exposure level was very close to the saturation concentration. Survival of the yeast cells was not adversely affected.

Conclusion

The tests performed failed to provide evidence of genetic activity that SEX may have in bacterial DNA repair or mutation tests or in yeast mitotic recombination assays.

HEXAHYDRO-1,3-DINITRO-5-ACETYL-S-TRIAMINE (TAX)

E.coli DNA Repair Tests on Plates (Table 11)

TAX was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no sign of toxicity in either strain in either the absence or presence of S-9 mix.

E.coli DNA Repair Tests in Suspension (Tables 12 and 13)

The highest exposure level used was 12.5 mg TAX.ml^{-1} incubation mixture. At this concentration precipitation occurred, but there was no toxicity in either strain in either the absence or presence of S-9 mix.

S. typhimurium Mutation Tests (Tables 14-16)

Preliminary toxicity tests with strain TA 98 indicated that TAX was not toxic to the bacteria, even at the very high exposure level of 10 mg per plate (Table 14). TAX remained in solution at this exposure level.

The mutagenicity tests were performed using S. typhimurium strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 15 and 16). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals between 10 µg and 10 mg per plate.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 17-20)

Toxicity tests were conducted in the absence and presence of S-9 mix using exposure levels up to 15 mg.ml^{-1} incubation mixture for 2 h. There were no indications of toxicity under any of these conditions (Table 17).

Tests for recombinogenic activity were performed using method 1 incubation conditions in the absence of S-9 mix only. There was no indication that TAX possessed recombinogenic potential. The positive control substance, EMS, on the other hand, induced high levels of recombination at an exposure level of 20 mg.ml^{-1}.

Using method 2 incubation conditions in the presence of S-9 mix, again there was no evidence of recombinogenic potential, there being no increase in the frequency or number of recombinants recovered over the exposure range 78 µg.ml^{-1} to 5 mg.ml^{-1} (Table 20). Survival was not adversely affected by this treatment.

Conclusion

The tests performed failed to provide evidence of genetic activity that TAX may have in bacterial DNA repair or mutation tests or in yeast mitotic recombination assays.

ETHYL CENTRALITE

E. coli DNA Repair Tests on Plates (Table 21)

Ethyl centralite was tested on plates at an exposure level of 10 mg per plate. There was no sign of toxicity in either strain in either the absence or presence of S-9 mix.

E. coli DNA Repair Tests in Suspension (Tables 22 and 23)

The highest exposure level used was 15.4 $mg.ml^{-1}$ incubation mixture. Precipitation first occurred at the 1.54 $mg.ml^{-1}$ exposure level, but there was no toxicity in either strain in either the absence or the presence of S-9 mix.

S. typhimurium Mutation Tests (Tables 24-26)

Preliminary toxicity tests with strain TA 98 indicated that ethyl centralite was not toxic to the bacteria, but precipitated in the culture medium at an exposure level of 3.3 mg per plate (Table 24).

The mutagenicity tests were performed using S. typhimurium strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 25 and 26). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals up to 3.3 mg per plate where there was toxicity, particularly in the absence of S-9 mix. Ethyl centralite precipitated at 1.0 mg and 3.3 mg per plate.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 27-34)

Ethyl centralite was dissolved in DMSO. Since it was considered desirable to limit the concentration of DMSO in the final incubation mixture to 10%, the highest concentrations tested for toxicity were determined by the solubility of ethyl centralite in the ultimate 10% DMSO, 90% water mixture. The highest concentration tested was, therefore, 41.7 $mg.ml^{-1}$. Incubations were for 30 min at 28°C (Tables 27-30). The toxicity test was repeated at 3 concentration levels and 5 time periods, the most severe exposure conditions being 44.2 $mg.ml^{-1}$ for 3 h (Table 29). This second toxicity test indicated that ethyl centralite might not be toxic in the presence of S-9 mix, but there were inconsistencies in the results making a repetition of the study desirable. Results of the third toxicity test again suggested that there may be a maximum toxic effect at an intermediate concentration level (Table 30). This rather odd result was viewed with scepticism, but was substantiated in part by the results of the recombination

assay using the method 1 conditions (Tables 32 and 33). There were no indications of recombinogenic activity in these experiments. The very high frequency of total aberrations was accompanied by survival being reduced to 1.5%, which must always lessen the significance of such a result.

When method 2 incubation conditions were adopted, the toxicity test had to be repeated. The results suggested that a high exposure level of 1 $mg.ml^{-1}$ be used (Tables 31 and 34). The unusal pattern of toxicity was repeated both in the toxicity test and the recombinogenic activity test. No evidence for recombinogenic potential was demonstrated.

Conclusion

Ethyl centralite did not induce genetic damage which was detected in any of the experiments performed.

Toxicity to the yeast cells was of an unusual pattern in that intermediate exposure conditions produced higher toxicity than either milder or more severe conditions.

2-NITRODIPHENYLAMINE

E. coli DNA Repair Tests on Plates (Table 35)

2-Nitrodiphenylamine was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no sign of toxicity in either strain in either the absence or presence of S-9 mix.

E. coli DNA Repair Tests in Suspension (Tables 36 and 37)

The highest exposure concentration used was 15.4 $mg.ml^{-1}$ incubation mixture. Precipitation first occurred at the 512 $\mu g.ml^{-1}$ exposure level, but there was no differential toxicity indicative of specific DNA damage.

S. typhimurium Mutation Tests (Tables 38-40)

Preliminary toxicity tests with strain TA 98 indicated that 2-nitrodiphenylamine was toxic to the bacteria and precipitated at an exposure level of 1.0 mg per plate (Table 38).

The mutagenicity tests were performed using S. typhimurium strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 39 and 40). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals up to 3.3 mg per plate. There was some toxicity towards the strains.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 41-45)

Toxicity tests were conducted in the absence and presence of S-9 mix at exposure concentrations up to 55.8 $mg.ml^{-1}$ incubation mixture using incubation method 1. There was no sign of a toxic effect over the 2 h incubation period. Using method 2 there was some loss of viability over the 18 h incubation period at an exposure concentration of 13.9 $mg.ml^{-1}$.

Tests for recombinogenic activity were performed using the method 1 incubation conditions in the absence of S-9 mix only. 2-Nitrodiphenylamine was not recombinogenic up to an exposure level of 52.5 $mg.ml^{-1}$. The compound precipitated in the incubation mixture at all the concentrations tested (the objective of such extreme testing being to examine for the possible presence of minor, recombinogenic, soluble contaminants in the 2-nitrodiphenylamine sample). Survival was unaccountably low at the 16 $mg.ml^{-1}$ concentration. This event, coupled with a high

number of hairline colonies produced a high frequency of aberrant colonies. Only one recombinant was recovered at this exposure level.

Method 2 incubation, in the presence of S-9 mix did not induce mitotic recombinants detectable in this assay.

Conclusion

2-Nitrodiphenylamine did not induce genetic damage which was detected in any of the experiments performed.

LEAD SALICYLATE

E. coli DNA Repair Tests on Plates (Table 46)

Lead salicylate was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no preferential toxicity to the polymerase-deficient strain in either the absence or presence of S-9 mix. According to the protocol, it was not necessary to proceed to testing in suspension as toxicity had been achieved on plates.

S. typhimurium Mutation Tests (Tables 47-49)

Preliminary toxicity tests with strain TA 98 indicated that lead salicylate was toxic and precipitated at an exposure level of 3.3 mg per plate (Table 47).

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 48 and 49). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals up to 3.3 mg per plate. At this exposure level and at 1 mg per plate, there was precipitation of the test compound and toxicity which was particularly evident for the mutant (i.e. colony-forming) bacteria.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 50-54)

Toxicity tests were conducted in the absence and presence of S-9 mix at exposure concentrations up to 53.1 $mg.ml^{-1}$ incubation mixture using incubation method 1. Lead salicylate clearly was toxic at much lower levels, so, the tests were re-run using incubation method 2. Toxicity was marked at 8 $mg.ml^{-1}$ in the presence of S-9 mix.

In the first recombinogenic test without S-9 mix, there was a suggestion that lead salicylate might induce mitotic recombination at exposure concentrations around 4 $mg.ml^{-1}$ (Table 52). The experiment was repeated at higher concentrations of test material. Survival was reduced to 79.5% at 10 $mg.ml^{-1}$ and 2.4% at 15 $mg.ml^{-1}$, but at neither of these exposure concentrations was there any indication of recombinogenic activity.

When S-9 mix was present and incubation method 2 used, viability was reduced to less than 1% at the 2 $mg.ml^{-1}$ exposure concentration. At the 3 lower exposure levels where viability was good, there was no indication of recombinogenic activity.

Conclusion

Lead salicylate did not induce genetic damage that was detected in any of the experiments performed.

LEAD RESORCYLATE

E. coli DNA Repair Tests on Plates (Table 55)

Lead resorcylate was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no preferential toxicity to the polymerase-deficient strain in either the absence or presence of S-9 mix. According to the protocol, it was not necessary to proceed to testing in suspension as toxicity had been achieved on plates.

S. typhimurium Mutation Tests (Tables 56-58)

Preliminary toxicity tests with strain TA 98 indicated that lead resorcylate was slightly toxic and precipitated at an exposure level of 3.3 mg per plate.

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 57 and 58). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals up to 10 mg per plate. There was precipitation at 3.3 mg per plate and specific toxicity to mutants at an exposure level of 10 mg per plate.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 59-64)

Toxicity tests conducted in the presence and absence of S-9 mix with up to 150 min exposures demonstrated that lead resorcylate was toxic at 40.5 $mg.ml^{-1}$ (Table 59). Method 2 incubation in the presence of S-9 mix indicated that lead resorcylate precipitated at 2 $mg.ml^{-1}$ but toxicity was not marked until 8 $mg.ml^{-1}$ (Table 60).

Recombinogenic tests without S-9 mix failed to demonstrate any activity under conditions of exposure where toxicity was mild to severe (Tables 61 and 62). Similarly, in the presence of S-9 mix there was no evidence of recombinogenic potential, although the positive control substance, cyclophosphamide, also was ineffective.

Conclusions

Lead resorcylate did not induce genetic damage that was detected in any of the experiments performed.

DIETHYLENEGLYCOLDINITRATE

E. coli DNA Repair Tests on Plates (Table 65)

Diethyleneglycoldinitrate was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no preferential toxicity to the polymerase-deficient strain in either the absence or presence of S-9 mix. According to the protocol, it was not necessary to proceed to testing in suspension as toxicity had been achieved on plates.

S. typhimurium Mutation Tests (Tables 66-68)

Preliminary toxicity tests with strain TA 98 indicated that diethyleneglycoldinitrate was not toxic to these bacteria, but that it precipitated in the incubation medium at an exposure level of 10 mg per plate (Table 66).

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 67 and 68). There was no sign of mutagenic activity in any of these strains either in the absence or presence of the S-9 mix, using exposure levels at half-log intervals up to 10 mg per plate. There was precipitation at this high level. The results with strain TA 98 were rather variable, but did not give rise to suspicion of a mutagenic response.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 69-72)

Toxicity tests conducted in the presence and absence of S-9 mix with up to 150 min exposures did not demonstrate any toxicity of diethyleneglycoldintrate at exposure concentrations as high as 135.5 mg.ml^{-1} (Table 69). Method 2 incubation toxicity test also failed to show any growth inhibiting potential (Table 70).

The recombinogenic tests did not reveal any potential for mitotic recombinogenic activity, either in the presence or absence of S-9 mix. While EMS was effective as a positive control, cyclophosphamide was not.

Conclusions

Diethyleneglycoldinitrate did not induce genetic damage detected in any of the experiments performed.

TETRYL

E. coli DNA Repair Tests on Plates (Table 73)

Tetryl was tested on plates at an exposure level of 10 mg per plate. Precipitation occurred, but there was no preferential toxicity to the polymerase-deficient strain in either the presence or absence of S-9 mix. According to the protocol, it was not necessary to proceed to testing in suspension as toxicity had been achieved on plates.

S. typhimurium Mutation Tests (Tables 74-77)

Preliminary toxicity tests with strain TA 98 indicated that tetryl might be mutagenic, particularly in the absence of S-9 mix and was toxic at an exposure level of about 333 μg per plate (Table 74).

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 75, 76, 77). The compound was toxic at 333 μg per plate and showed strong evidence of mutagenic activity detected with strains TA 1537, TA 1538, TA 98 and TA 100. Because of inconsistencies in the first experimental results with TA 98, this portion of the test was repeated, but the second set of figures confirmed the impression that tetryl is a mutagen.

Activity was diminished in the presence of S-9 mix, but it is not known whether this was truely a deactivation reaction or simply competitive inhibition of tetryl reactions with bacterial DNA by the large amount of protein and RNA present in the S-9 mix.

The most sensitive strain was the frameshift indicator strain TA 1537 and the least effective exposure level in this strain was less then 1 μg per plate.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 78-85)

Toxicity tests conducted in the presence and absence of S-9 mix with up to 150 min exposures did demonstrate toxicity of tetryl within 1 h of incubation at a concentration of 19.9 $mg.ml^{-1}$ (Table 78). The toxicity test in the absence of S-9 mix was repeated (Table 79). Survival was low again, being approximately 2% at a tetryl concentration of 125 $\mu g.ml^{-1}$. Eighteen hour incubation (method 2) in the presence of S-9 mix also resulted in low survival at a concentration of 125 $\mu g.ml^{-1}$ (Table 80).

The first recombinogenic test in the absence of S-9 mix gave very few survivors at any exposure concentration (Table 81), but a more satisfactory level of survival was obtained in the second test (Table 82). Mitotic recombinant and total aberrant frequencies were increased in this experiment, but the absolute numbers involved were low. It was necessary, therefore, to confirm or refute the suspicion of a positive response. Recombinant and total aberrant frequencies were again increased, but once more the absolute numbers were low (Table 83).

In order to make comparison between control and treated cells more reliable, the experiment was repeated a third time without S-9 mix, but the technique for plating out the cells was altered. Using haemacytometer counts as a guide, approximately equal numbers of cells were plated in the treated groups as in the controls. Consequently, 100 plates were assessed in the vehicle, control group, 200 plates in the 62.5 $\mu g.ml^{-1}$ group and 600 plates in the 125 $\mu g.ml^{-1}$ group (Table 84). The results show quite clearly that tetryl induces mitotic recombination and other aberrations in yeast cells, in the absence of S-9 mix.

Method 2 incubation of tetryl with S-9 mix did not induce any significant change in recombinant or total aberrant cell numbers or frequencies (Table 85).

Analytical Quality of Tetryl

In view of the demonstration of genetic damage induced by tetryl, the sample was analysed and the Certificate of Analytical Quality follows (p. 34). The assumption was made that the minor impurities detected by HPLC had the same extinction coefficient as tetryl. On this basis, the minimum purity of the tetryl sample was 99.6%. The impurities were not identified.

Conclusions

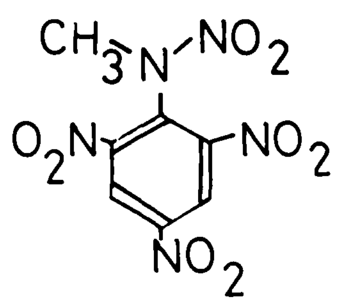
Tetryl did not induce DNA damage in *E. coli* which was repairable by the deficient DNA polymerase. On the other hand, mutagenic responses were obtained with *S. typhimurium* TA 1537, TA 1538, TA 98 and TA 100 which were particularly strong in the absence of S-9 mix. Recombinogenic activity was also demonstrated in the absence of S-9 mix in 3 independent experiments. The presence of S-9 mix did not permit this activity to be seen.

INVERESK RESEARCH INTERNATIONAL

PROJECT NO.: 410110

CERTIFICATE OF ANALYTICAL QUALITY

Tetryl
$C_7H_5N_5O_8$
M.W. = 287.157



Source: M.O.D. Waltham Abbey, England

Batch No. -

Date obtained: - 14th November 1978

Storage location: Mutagenesis Laboratory, IRI

IRI Notebook reference: 410110/1

Names of Analysts: (i) M.S. Henderson

(ii) J.N. Done

(iii) P. Teale

Analytical Data checked by: (i) [illegible] (M.S).

(ii) [illegible]

Analytical Certificate issued by:

Inveresk Research International
Edinburgh

Date: 28 [illegible] 1979

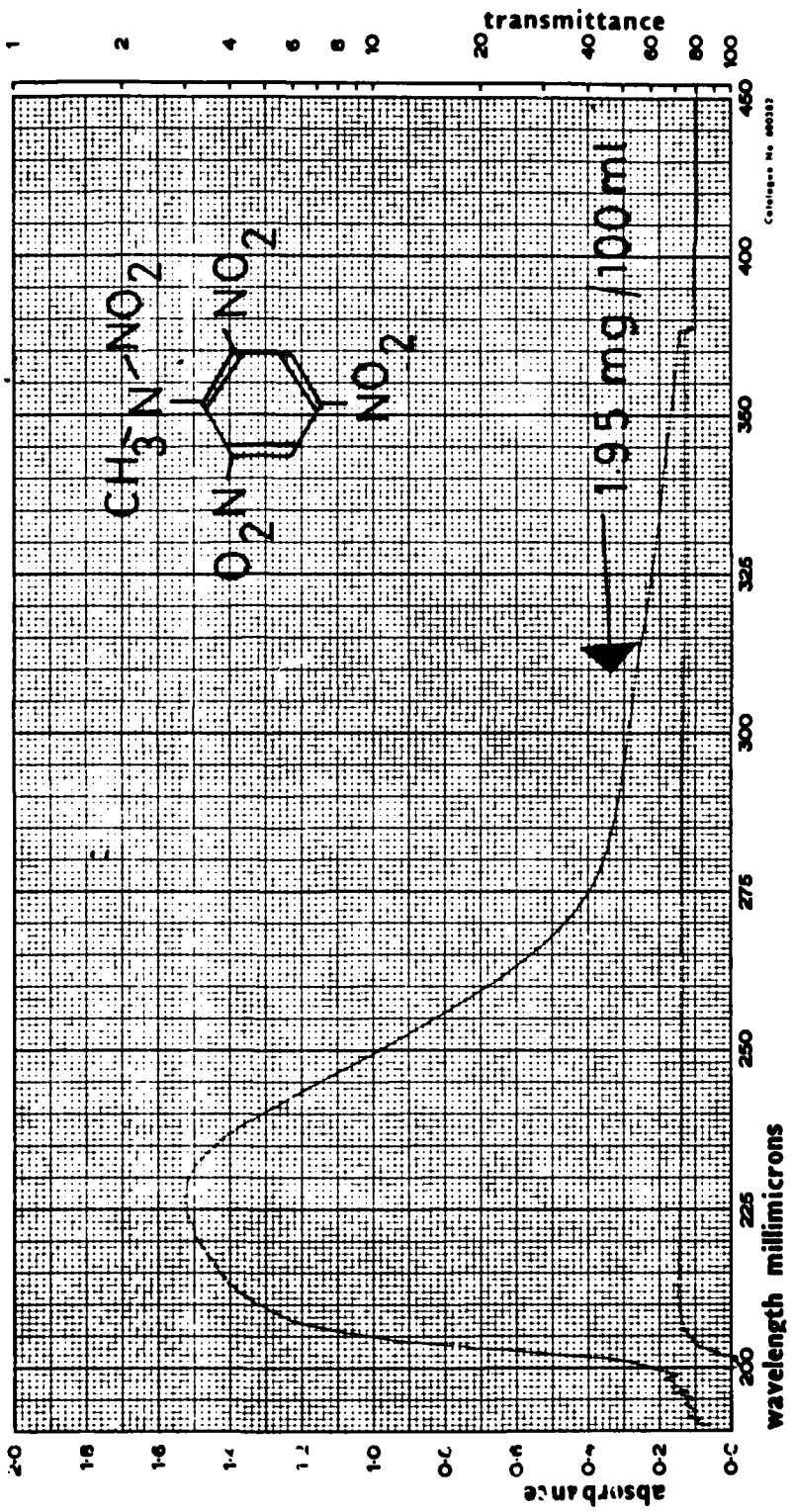
Certificate of Analytical Quality (Tetryl)

<table>
<tr><th>TEST</th><th>RESULTS AND COMMENTS</th></tr>
<tr><td>Physical appearance</td><td>Yellow amorphous powder; no detectable odour</td></tr>
<tr><td>Melting point</td><td>Found: 128-130°C (uncorrected)
Reported: 130-132°C (Merck Index)</td></tr>
<tr><td>Solubility data</td><td>Found: Readily soluble (10% solution) in acetone, dimethyl sulphoxide, dimethyl formamide; sparingly soluble in hot chloroform, methanol and ethanol; insoluble in water.</td></tr>
<tr><td>Elemental analysis</td><td>
<table>
<tr><td>Element</td><td>C</td><td>H</td><td>N</td><td></td></tr>
<tr><td>Calculated:</td><td>29.28</td><td>1.76</td><td>24.39</td><td></td></tr>
<tr><td>Found (i) :</td><td>30.34</td><td>1.93</td><td>24.56</td><td></td></tr>
<tr><td>(ii):</td><td>30.10</td><td>1.90</td><td>24.39</td><td></td></tr>
</table>
</td></tr>
<tr><td>Ultraviolet/ visible spectrum</td><td>Spectrum attached: 1.95 mg/100 ml methanol: methanol in reference beam: 10 mm quartz cells: Unicam SP800 spectrophotometer.
<table>
<tr><td>λ_{max}, nm</td><td>227</td><td></td><td></td><td></td></tr>
<tr><td>log ε</td><td>4.31</td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>Infrared spectrum</td><td>Spectrum attached: KBr disc: Perkin Elmer 257 spectrometer.</td></tr>
</table>

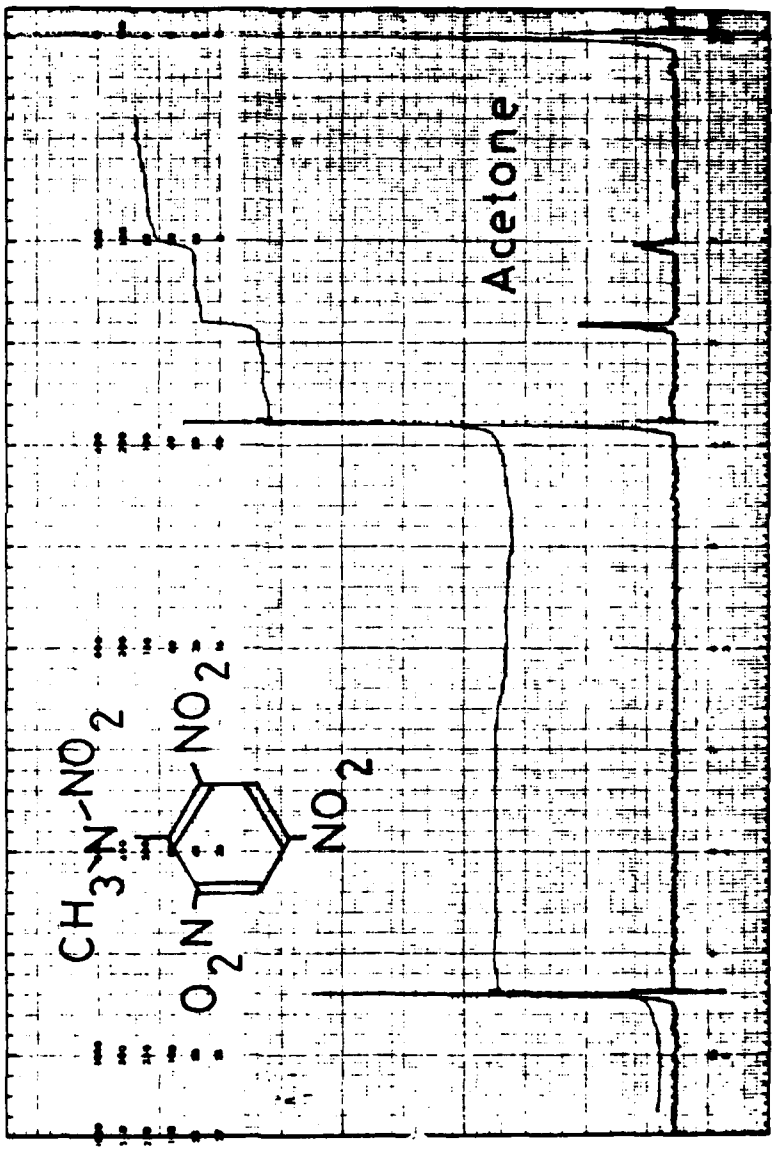
Certificate of Analytical Quality (Tetryl)

<table>
<tr><td>Proton magnetic resonance spectrum</td><td>Spectrum attached. In d_6 acetone solution On Varian Associates HA-100 spectrometer.
<table>
<tr><th>δ</th><th>Chemical moiety</th><th>No. protons by integration</th></tr>
<tr><td>9.4</td><td>Aromatic - H</td><td>2</td></tr>
<tr><td>3.78</td><td>N-CH_3</td><td>3</td></tr>
</table></td></tr>
<tr><td>Thin-layer chromatography</td><td>Silica gel G (0.25 mm): In(i) chloroform (ii) ethyl acetate - light petroleum (1:3 v/v) iodine vapour and ultraviolet (254 nm) detection. (i) 500, 1000, 1500 µg applied; major spot Rf = 0.31, trace spot Rf = 0.51. (ii) 200, 400, 600 µg applied; single spot Rf = 0.26.</td></tr>
<tr><td>High performance liquid chromatography</td><td>Varian 8500 liquid chromatograph 250 x 5 mm Hypersil ODS:U.v. detection 235 nm; mobile phase acetonitrile:water (2:3 v/v) 1.5 ml/min; chart speed 600 mm/h.</td></tr>
<tr><td>Gas-liquid chromatography</td><td>-</td></tr>
<tr><td>Mass spectrum</td><td>Finnigan 4000 GC-MS (6110 Data System) Source 260°C (indicated); EI; 70eV Mass Spectrum attached.</td></tr>
<tr><td>Other analytical methods</td><td>-</td></tr>
<tr><td>General comments</td><td>(i) Elemental analyses were performed by Butterworth Laboratories Ltd, Teddington, Middlesex.
(ii) Assuming the impurities detected by the HPLC high load chromatography (peaks A & B) have the same extinction coefficients as the Tetryl this corresponds to about 0.35% of the total sample.</td></tr>
</table>

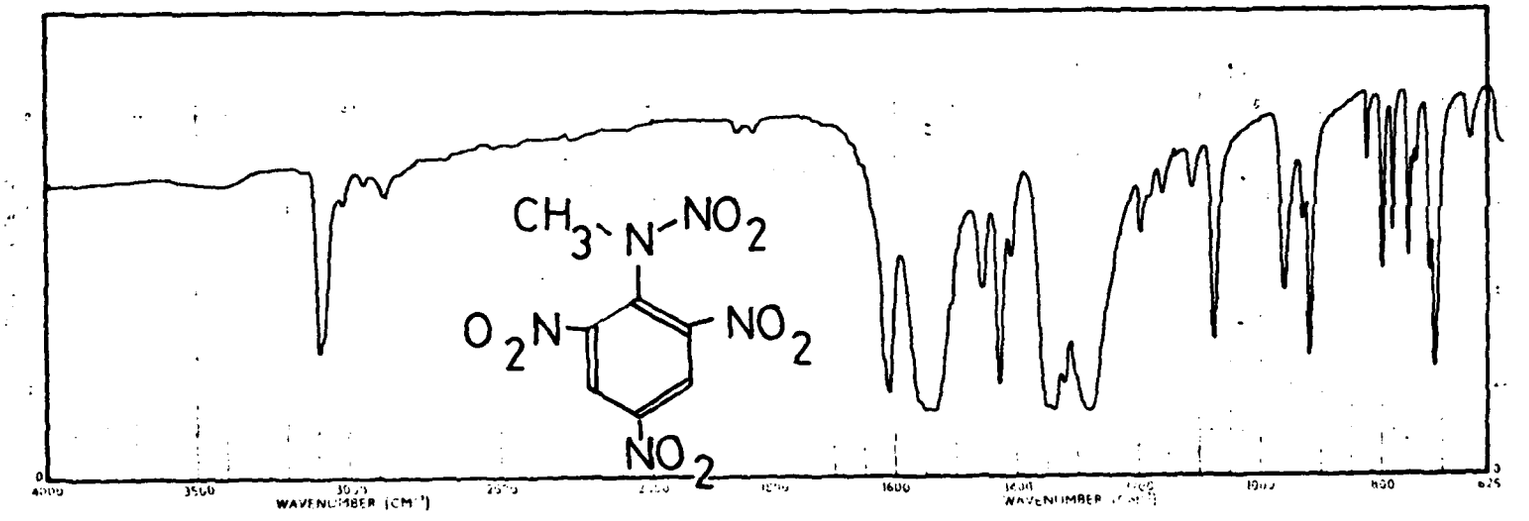
Ultraviolet spectrum of tetryl



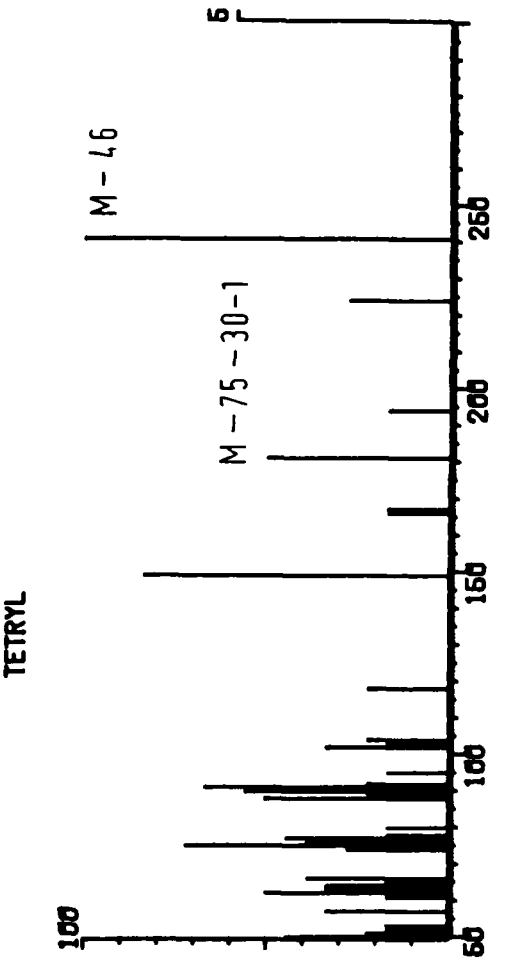
Nuclear magnetic resonance spectrum of tetryl

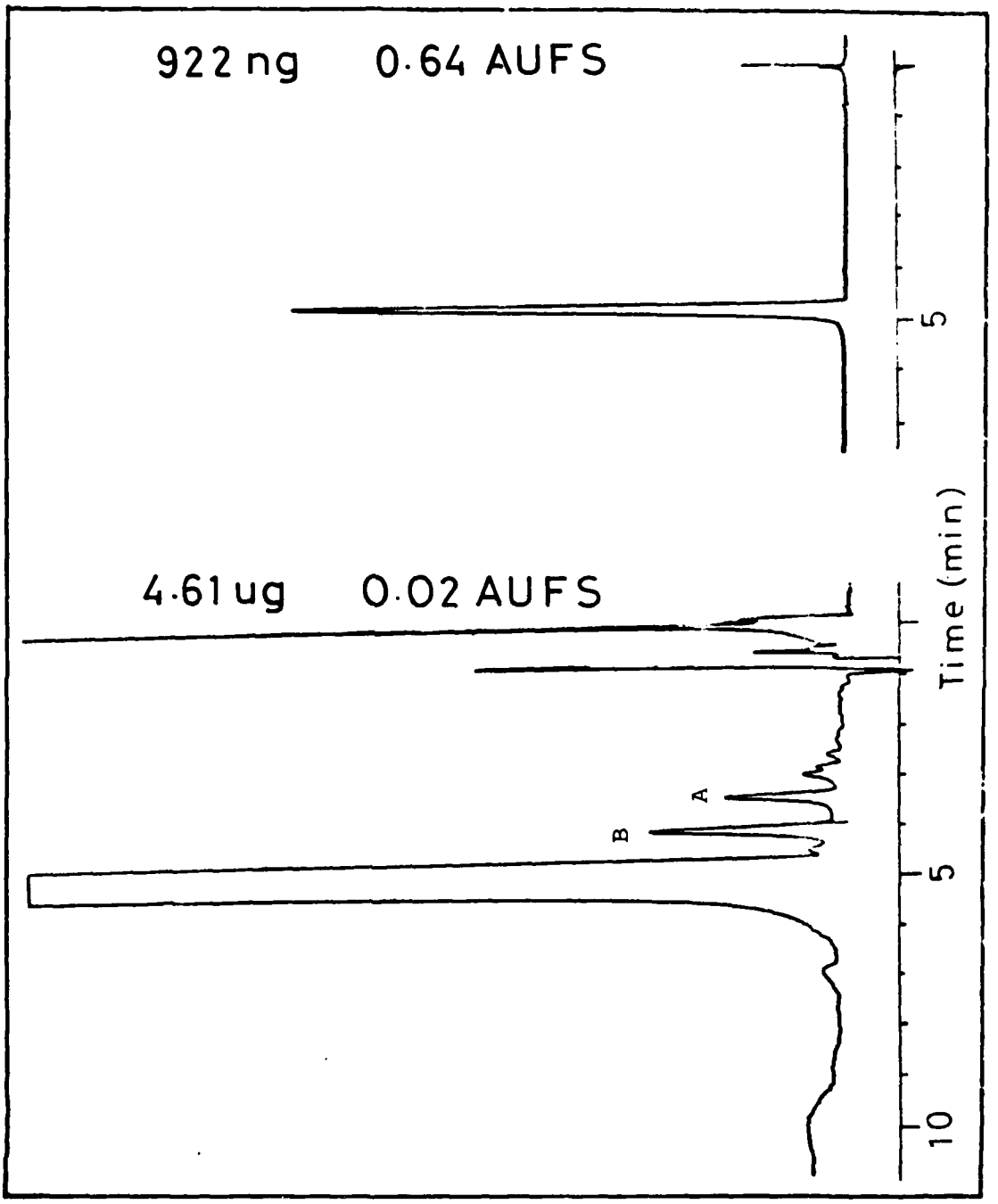


Infrared spectrum of tetryl



Mass spectrum of tetryl





High performance liquid chromatogram of tetryl

RED PHOSPHORUS

E. coli DNA Repair Test on Plates (Table 86)

Red phosphorus was tested on plates at an exposure level of 10 mg per plate. There was no sign of a toxic effect, therefore, it was tested in suspension.

E. coli DNA Repair Test in Suspension (Tables 87 and 88)

When tested to the very high exposure level of 15.4 $mg.ml^{-1}$, red phosphorus showed no signs of being toxic to the bacteria either in the absence or presence of S-9 mix.

S. typhimurium Mutation Tests (Tables 89-91)

Preliminary toxicity tests with strain TA 98 indicated that red phosphorus was not toxic to this strain at exposure levels up to 10 mg per plate (Table 89).

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 90 and 91). There was no sign of mutagenic activity in any of these strains either in the absence or presence of S-9 mix, using exposure levels at half-log intervals up to 10 mg per plate.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 92-95)

Toxicity tests conducted in the presence and absence of S-9 mix with up to 150 min exposures did not demonstrate any toxicity of red phosphorus (Table 92). A similar result was obtained using method 2 incubation conditions in the presence of S-9 mix for 18 h and an exposure concentration of 10 $mg.ml^{-1}$ (Table 93).

No evidence for recombinogenic activity was obtained when red phosphorus was incubated with yeast cells either in the absence of S-9 mix (method 1, Table 94) or in its presence (method 2, Table 95).

Conclusion

The tests conducted did not provide evidence that red phosphorus induced genetic damage in bacteria or yeast cells.

NITROGUANIDINE

E. coli DNA Repair Tests on Plates (Table 96)

Nitroguanidine was tested on plates at an exposure level of 10 mg per plate. There was no preferential toxicity to the polymerase-deficient strain in either the presence or absence of S-9 mix. According to the protocol, it was not necessary to proceed to testing in suspension as toxicity had been achieved on plates.

S. typhimurium Mutation Tests (Tables 97-99)

Preliminary toxicity tests with strain TA 98 indicated that nitroguanidine was not toxic to the bacteria at exposure levels up to 10 mg per plate.

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 98 and 99). There was no indication of a mutagenic response in any strain in the presence or absence of S-9 mix.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 100-103)

Toxicity tests were conducted in the presence and absence of S-9 mix, but there was no clear indication that nitroguanidine was toxic to the yeast cells over a 150 min incubation period at a concentration of 22.7 mg.ml^{-1} (Table 100). Method 2 incubation conditions in the presence of S-9 mix for 18 h also failed to induce toxicity (Table 101).

Recombinogenic activity tests without S-9 mix (Table 102) and with S-9 mix (Table 103) did not show any significant response to nitroguanidine treatment.

Conclusion

Nitroguanidine did not induce genetic damage which was detected in these tests for DNA repair or mutation induction in bacteria or recombinogenic activity in yeast.

N-NITROSODIPHENYLAMINE

E. coli DNA Repair Tests on Plates (Table 104)

N-Nitrosodiphenylamine was tested on plates at an exposure level of 10 mg per plate. These experimental conditions did not induce toxicity in either strain.

E. coli DNA Repair Tests in Suspension (Tables 105-108)

When tested to the very high exposure concentration of 15.4 $mg.ml^{-1}$, no preferential toxicity was found either in the absence of S-9 mix (Table 105) or in its presence (Table 107). In both of these experiments there were factors manifest in the results indicating that re-testing was necessary. These re-tests (Tables 106 and 108) confirmed the initial findings.

S. typhimurium Mutation Tests (Tables 109-111)

Preliminary toxicity tests with strain TA 98 indicated that N-nitrosodiphenylamine was toxic to the bacteria at an exposure level of 10 mg per plate. Precipitation in the incubation medium occurred at an exposure level of 1 mg per plate.

The mutagenicity tests were performed using S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 (Tables 110 and 111). No mutagenic response was detected either in the presence or absence of S-9 mix.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 112-118)

Toxicity tests conducted in the presence and absence of S-9 mix showed that N-nitrosodiphenylamine was non-toxic to yeast cells over 150 min incubation time at concentration levels up to 52.7 $mg.ml^{-1}$ (Tables 112-114). A toxicity test using incubation method 2, in the presence of S-9 mix for 18 h, also showed that N-nitrosodiphenylamine was non-toxic (Table 115).

No recombinogenic activity was demonstrated (Tables 116 and 117). The compound precipitated at all concentration levels used, such exhaustive testing being conducted in order to detect possible active contaminants.

Conclusion

N-Nitrosodiphenylamine did not induce genetic damage which was detected in these tests for DNA repair or mutation induction in bacteria or recombinogenic activity in yeast.

DIPHENYLAMINE

E. coli DNA Repair Tests on Plates (Table 119)

Diphenylamine did not show preferential toxicity for the polymerase-deficient strain at an exposure level of 10 mg per plate either in the presence or absence of S-9 mix.

S. typhimurium Mutation Tests (Tables 120-122)

A preliminary toxicity test with strain TA 98 showed that diphenylamine was toxic at an exposure level of 333 µg per plate. Precipitation occurred at this exposure level (Table 120).

The mutagenicity tests were conducted with S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 in the presence and absence of S-9 mix. There was no evidence for a mutagenic response in any of these strains (Tables 121-122).

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 123-126)

Toxicity tests showed that diphenylamine was toxic to yeast cells at concentrations less than 250 $\mu g.ml^{-1}$ (Tables 123 and 124).

Recombinogenic tests, using exposure concentrations which extend to well within the toxic range, failed to detect any activity induced by the test compound.

Conclusion

Induction of genetic damage by diphenylamine was not demonstrated using DNA repair tests or mutation tests in bacteria or recombination tests in yeasts.

1,3-DINITROBENZENE

E. coli DNA Repair Tests on Plates (Table 127)

1,3-Dinitrobenzene did not show preferential toxicity for the polymerase-deficient strain at an exposure level of 10 mg per plate either in the presence or absence of S-9 mix.

S. typhimurium Mutation Tests (Tables 128-131)

A preliminary toxicity test with strain TA 98 showed that 1,3-dinitrobenzene was toxic at an exposure level of 1 mg per plate. There was also an indication in this test that the compound might be mutagenic (Table 128).

The mutagenicity tests were conducted with S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 in the presence and absence of S-9 mix (Tables 129-131). Mutagenic responses were obtained in strains TA 1538, TA 98 and TA 100. Significant, single dose level responses were also obtained with TA 1537, both with and without S-9 mix. The experiment with TA 98 was repeated over a narrower exposure level range and a good exposure-response relationship was established. There was a tendency for 1,3-dinitrobenzene to be active at a lower exposure level in the absence of S-9 mix than in the presence of this preparation.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 132-134)

Toxicity tests showed that 1,3-dinitrobenzene was not toxic to yeast when incubated with the cells over 150 min at concentrations up to 55.1 mg.ml^{-1} (Table 132). There was no indication for a recombinogenic response without S-9 mix (Table 133) or, using incubation method 2, in the presence of S-9 mix (Table 134).

Analytical Quality

In view of the mutagenic response obtained with 1,3-dinitrobenzene, it was considered desirable to authenticate the sample and establish its purity. The Certificate of Analytical Quality is given on p. 48. HPLC detection limits under the experimental conditions quoted were estimated for some potential impurities, but absolute detection limits for all potential impurities were not determined. The minimum purity of 1,3-dinitrobenzene was estimated to be 99.7%. On column detection limits for nitrobenzene and 1,3,5-trinitrobenzene were 2 ng and 9 ng respectively. Minor impurities detected by HPLC and TLC were not identified.

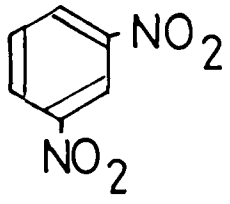
Conclusions

1,3-Dinitrobenzene is mutagenic and detectable with strains TA 1538, TA 98 and TA 100. S-9 mix appears to reduce the magnitude of the response. No other potential for inducing genetic damage was demonstrated with a bacterial DNA repair test and a yeast test for recombinogenic activity.

INVERESK RESEARCH INTERNATIONAL

PROJECT NO.: 410110

CERTIFICATE OF ANALYTICAL QUALITY

1,3-Dinitrobenzene
$C_6H_4N_2O_4$
M.W. = 168.114


Source: British Drug Houses Chemicals Ltd., Poole, England

Batch No. 6105910

Date obtained: 3rd November 1978

Storage location: Mutagenesis Laboratory - IRI

IRI Notebook reference: 410110/11

Names of Analysts: (i) M.S. Henderson

(ii) J.N. Done

(iii) P. Teale

Analytical Data checked by: (i) M.S. Henderson (M.S)

(ii) G.A. Byrne

Analytical Certificate issued by:

Inveresk Research International
Edinburgh

Date: 31 August 1979

Certificate of Analytical Quality (1,3-dinitrobenzene)

TEST	RESULTS AND COMMENTS
Physical appearance	White crystaline solid; no detectable odour
Melting point	Found: 86-88°C (uncorrected) Reported: 89-90°C (Merck Index)
Solubility data	Found: Soluble (10% solution) in chloroform, acetone, dimethyl sulphoxide, dimethyl formamide, soluble with heating in methanol and ethanol. Insoluble in light petroleum, hexane and water.

Elemental analysis

Element	C	H	N	
Calculated:	42.87	2.40	16.66	
Found (i) :	43.86	2.34	16.78	
(ii):	43.71	2.50	16.66	

Ultraviolet/ visible spectrum

Spectrum attached: 0.98 mg/100 ml methanol: methanol in reference beam: 10 mm quartz cells: Unicam SP800 spectrophotometer.

λ_{max}, nm	234			
log ε	4.22			

Infrared spectrum

Spectrum attached: KBr disc: Perkin Elmer 257 spectrometer.

Ref: J.H.S. Green & H.A. Lauwers Spectrochimica Acta, 27A, 817, 1971.

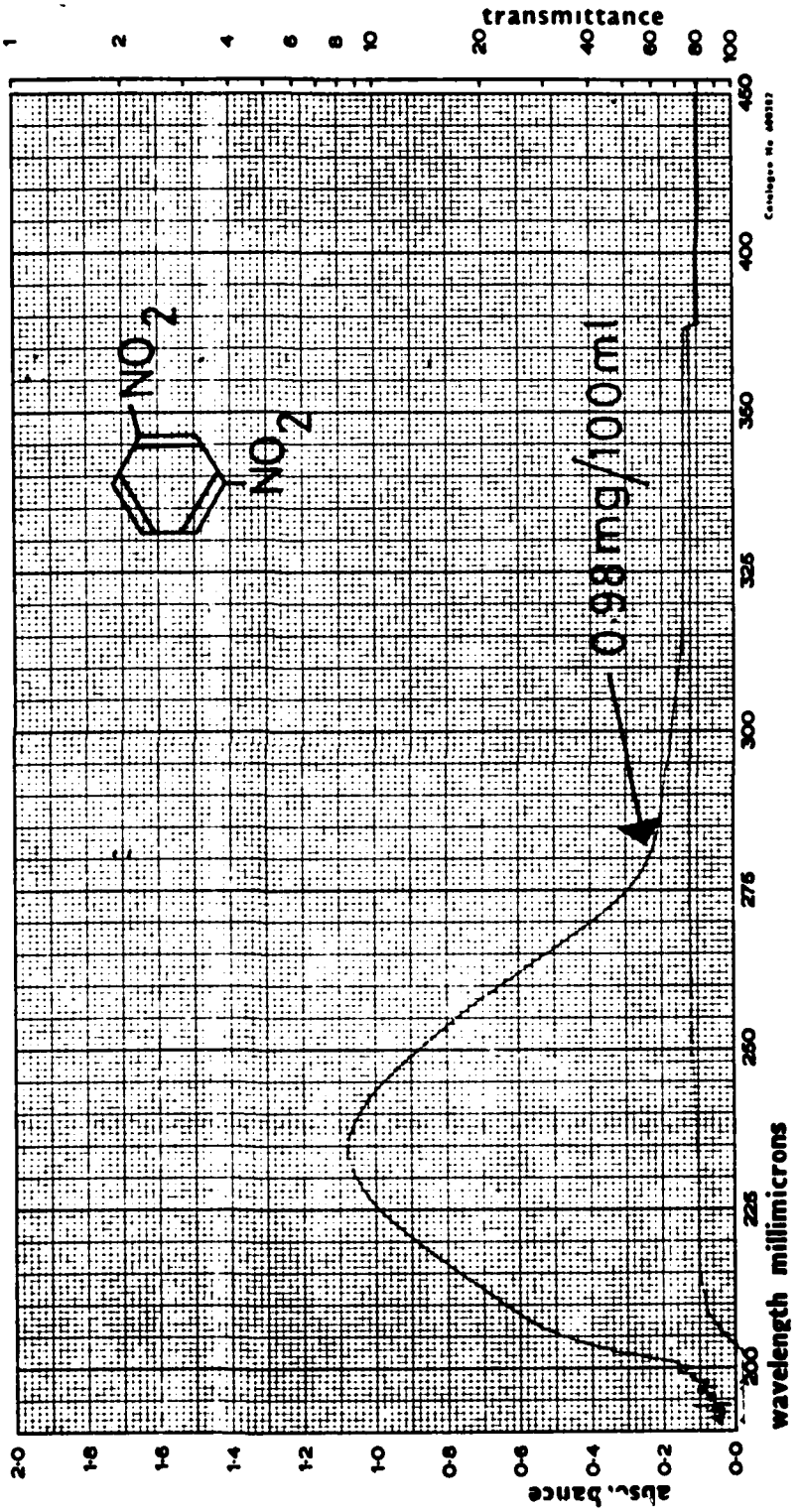
X, Y in $m\text{-}XC_6H_4Y$	NO_2, NO_2 Lr.*	NO_2, NO_2 R[9]	X, Y in $m\text{-}XC_6H_4Y$	NO_2, NO_2 Lr.*	NO_2, NO_2 R[9]
a_1 ν_1	3100	3100	b_1 ν_{21}	978	
ν_2	3075		ν_{22}	916	
ν_3	3050		ν_{23}	817	
ν_4	1601	1601	$\nu_{24}\gamma(NO_2)$	740	
$\nu_5\ \nu_{25}(NO_2)$	1545	1541			
ν_6	1440		b_2 ν_{29}	3095	
$\nu_7\ \nu_8(NO_2)$	1350	1353	ν_{30}	1614	
ν_8	1152	1150	$\nu_{31}\ \nu_{32}(NO_2)$	1528	
ν_9	1074	1070	ν_{32}	1465	
ν_{10}	1004	1001	$\nu_{33}\ \nu_9(NO_2)$	1368	1375
$\nu_{11}\ \delta(NO_2)$	836	840	ν_{34}	1310	1312
			ν_{35}	1278	~1280
a_2 ν_{16}	(900)		ν_{36}	1172	1174
$\nu_{17}\gamma(NO_2)$	760	763	ν_{37}	1096	~1095
			$\nu_{38}\ \delta(NO_2)$	806	809
			ν_{39}	725	

Certificate of Analytical Quality (1,3-dinitrobenzene)

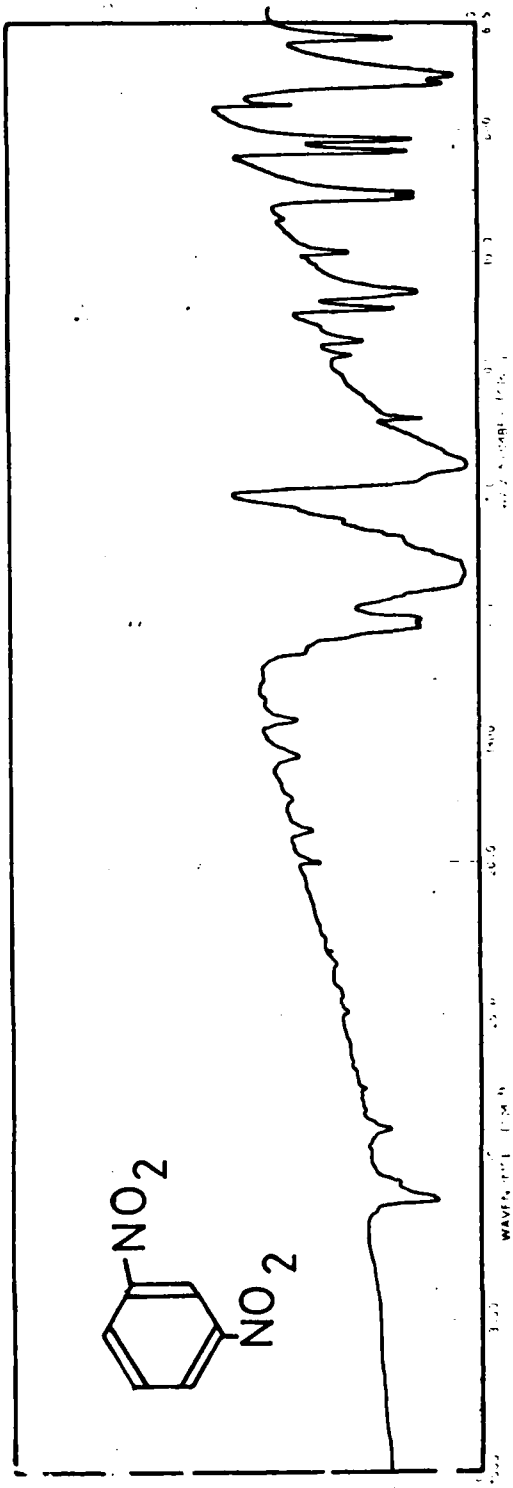
<table>
<tr><td>Proton magnetic resonance spectrum.</td><td>Spectrum attached. In d_6 acetone solution on Varian Associates HA100 spectrometer

<table><tr><th>δ</th><th>Chemical moiety</th><th>No. protons by integration</th></tr><tr><td>8.98
8.70
8.03</td><td>Aromatic -H
Aromatic -H
Aromatic -H</td><td>1
2
1</td></tr></table></td></tr>
<tr><td>Thin-layer chromatography</td><td>Silica gel G (0.25 mm): (i) chloroform (ii) ethylacetate-light petroleum (1:3 v/v) iodine vapour and ultraviolet (254 nm) detection.
500, 1000 and 1500 µg applied (both systems)
(i) single spot Rf = 0.52
(ii) single spot Rf = 0.32</td></tr>
<tr><td>High performance liquid chromatography</td><td>Varian 8500 liquid chromatograph
250 x 5 mm Hypersil silica:(i) 1% acetonitrile in n-hexane. 2 ml/min: 254 nm. Chart speed 300 mm/h. (ii) 1.5% acetonitrile in n-hexane</td></tr>
<tr><td>Gas-liquid chromatography</td><td>-</td></tr>
<tr><td>Mass spectrum</td><td>Finnigan 4000 GC-MS (6110 Data System)
Source 260°C (indicated); EI; 70eV
Mass Spectrum attached.</td></tr>
<tr><td>Other analytical methods</td><td>-</td></tr>
<tr><td>General comments</td><td>i) In order to show the absence of possible impurities, hplc of a mixture of mono-, di- & tri-nitrobenzenes has been undertaken. Chromatograms attached.
ii) Elemental analyses were performed by Butterworth Laboratories Limited, Teddington, Middlesex.</td></tr>
</table>

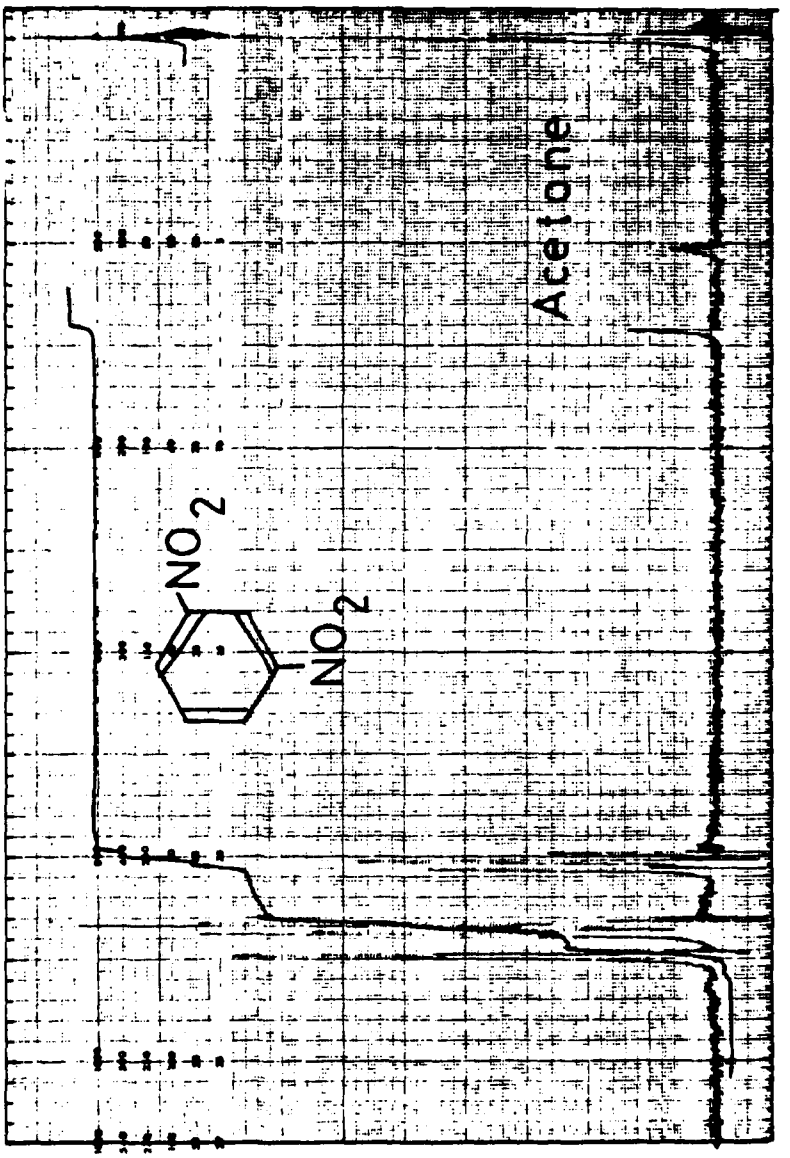
Ultraviolet spectrum of 1,3-dinitrobenzene



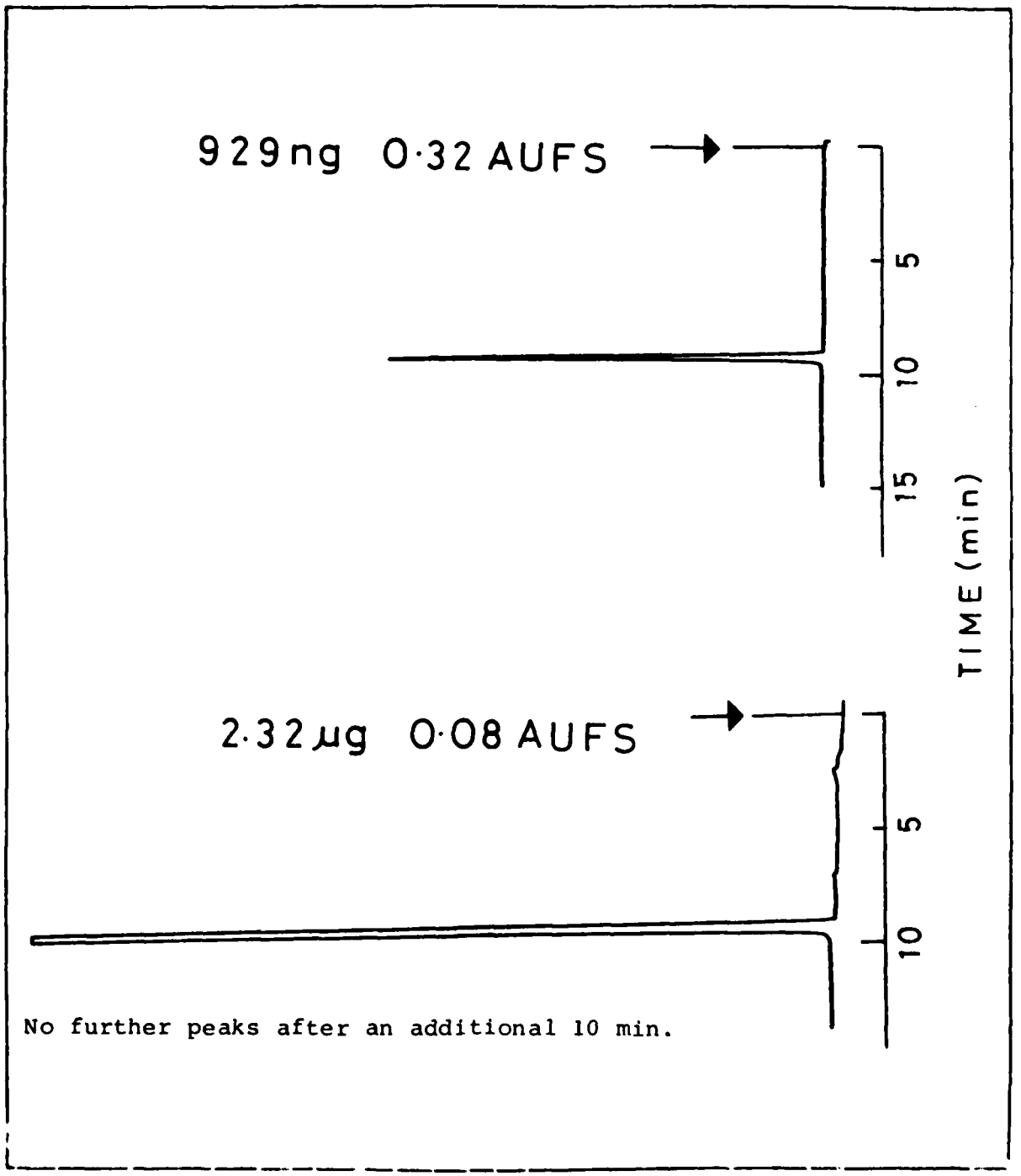
Infrared spectrum of 1,3-dinitrobenzene



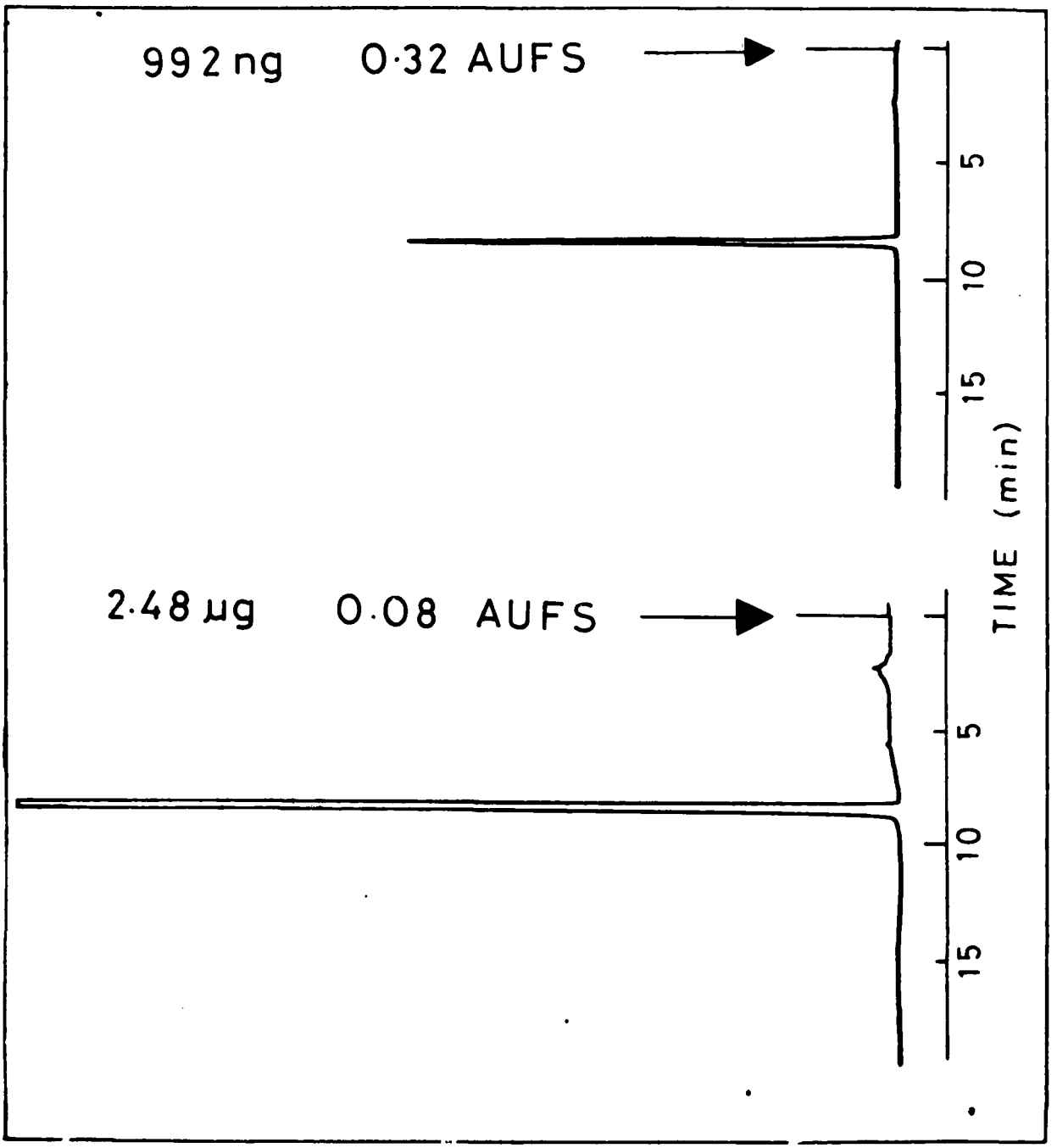
Nuclear magnetic resonance spectrum of 1,3-dinitrobenzene



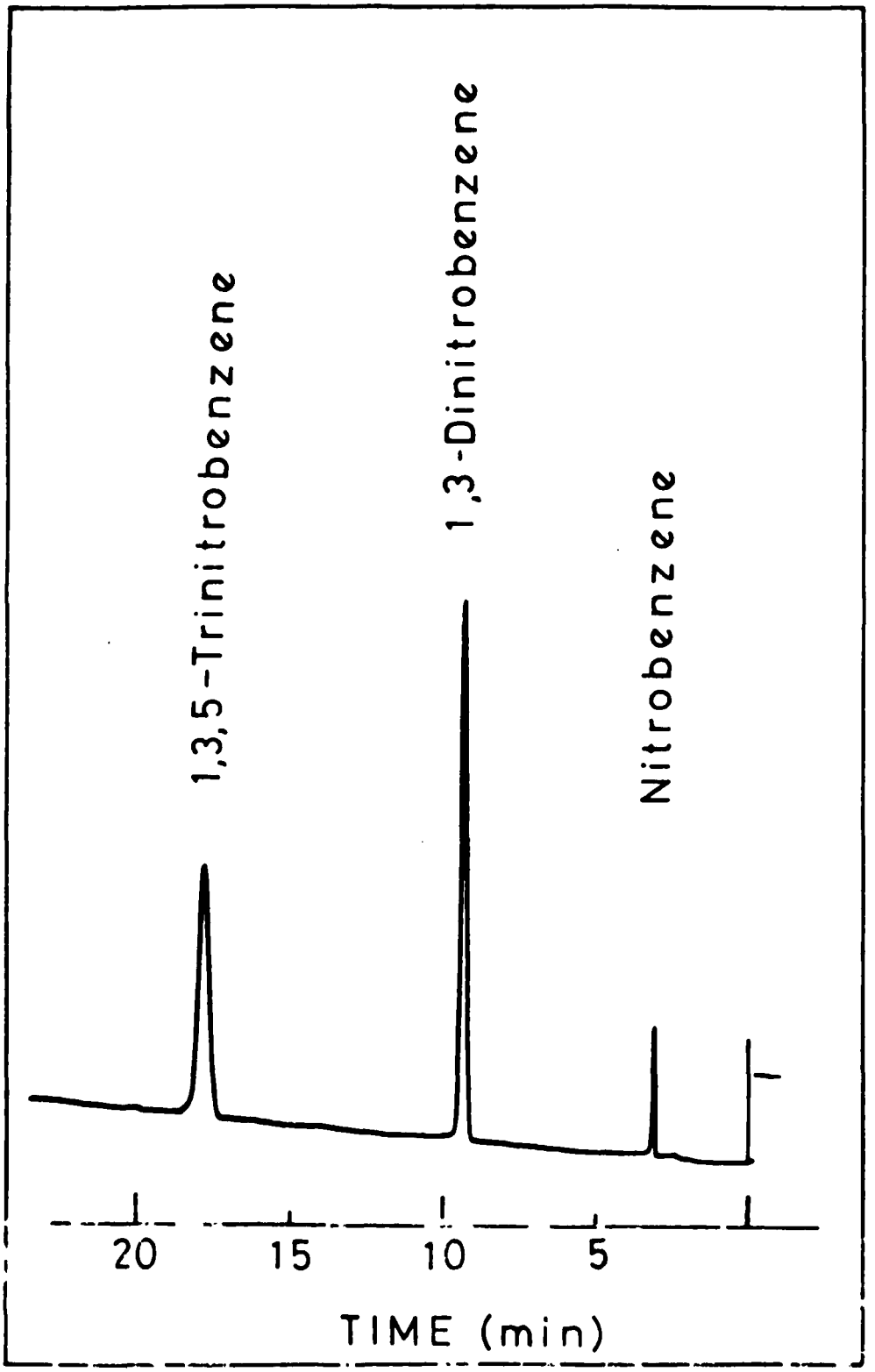
High performance liquid chromatogram of
1,3-dinitrobenzene using 1% acetonitrile in hexane



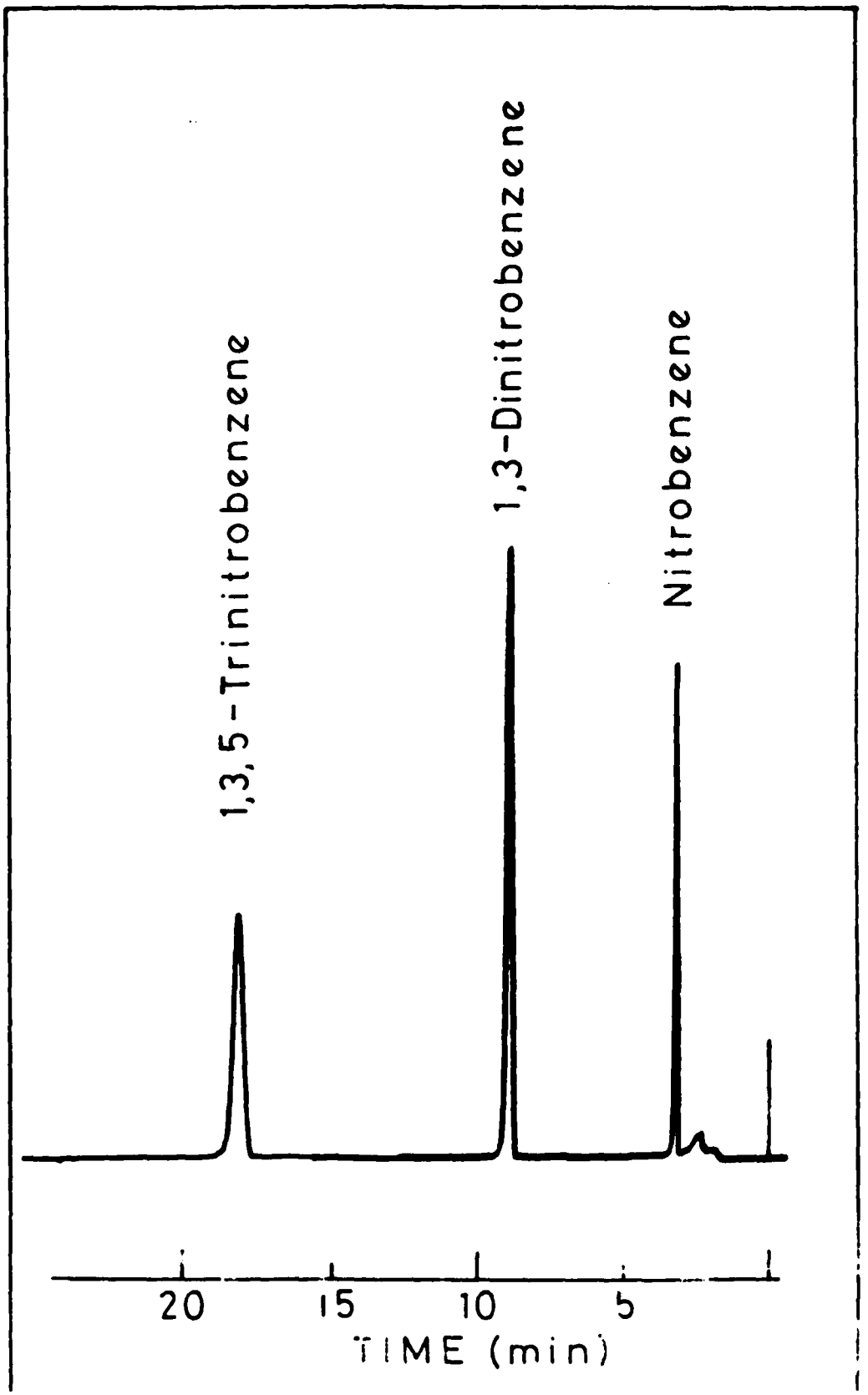
High performance liquid chromatogram of
1,3-dinitrobenzene using 1.5% acetonitrile in hexane



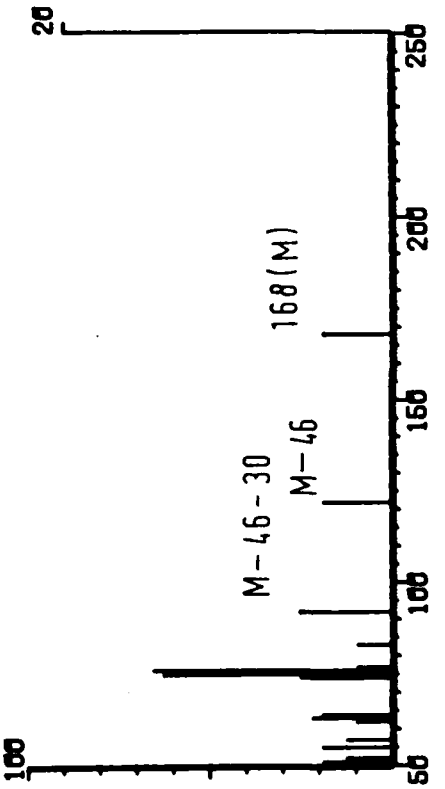
High performance liquid chromatogram of
the nitrobenzenes using 1% acetonitrile in hexane



High performance liquid chromatogram of
the nitrobenzenes using 1.5% acetonitrile in hexane



Mass spectrum of 1,3-dinitrobenzene



1,3,5-TRINITROBENZENE

E. coli DNA Repair Tests on Plates (Table 135)

1,3,5-Trinitrobenzene did not show preferential toxicity for the polymerase-deficient strain at an exposure level of 10 mg per plate either in the presence or absence of S-9 mix.

S. typhimurium Mutation Tests (Tables 136-138)

A preliminary toxicity test with strain TA 98 showed that 1,3,5-trinitrobenzene was toxic at an exposure level of 100 μg per plate. There was also an indication in this test that the compound might be mutagenic (Table 136).

The mutagenicity tests were conducted with S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 in the presence and absence of S-9 mix (Tables 137 and 138). Mutagenic responses were obtained in strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. There was a tendency for 1,3,5-trinitrobenzene to be active at a lower exposure level in the absence of S-9 mix than in the presence of this preparation.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 139-143)

Toxicity tests showed that 1,3,5-trinitrobenzene was not toxic to yeast when incubated with the cells over 150 min at concentrations up to 66.7 $mg.ml^{-1}$ (Table 139).

Using incubation method 2, in the presence of S-9 mix for 18 h, there was a gradual decline in survival from the lowest exposure concentration used (62.5 $\mu g.ml^{-1}$) (Table 140).

Recombinogenic tests were performed, but it was concluded that there was no potential for this activity (Tables 141-143). The first attempt at the recombinogenic test in the presence of S-9 mix was not followed through because cell survival was very low.

Analytical Quality

In view of the mutagenic response obtained with 1,3,5-trinitrobenzene, it was considered desirable to authenticate the sample and establish its purity. The Certificate of Analytical Quality is given on p. 61.

HPLC detection limits under the experimental conditions quoted were estimated for some potential impurities, but absolute detection limits for all potential impurities were not determined. The minimum purity of 1,3,5-trinitrobenzene

was 98.8%. On column detection limits for nitrobenzene and 1,3-dinitrobenzene were 2 ng and 4 ng respectively. Minor impurities detected by HPLC and TLC were not identified.

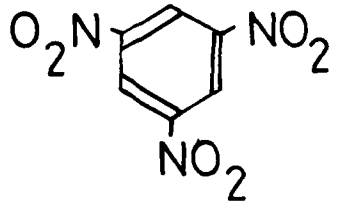
Conclusions

1,3,5-Trinitrobenzene is mutagenic and detectable with strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. S-9 mix appears to reduce the magnitude of the response. No other potential for inducing genetic damage was demonstrated with a bacterial DNA repair test and a yeast test for recombinogenic acticity.

INVERESK RESEARCH INTERNATIONAL

PROJECT NO.: 410110

CERTIFICATE OF ANALYTICAL QUALITY

1,3,5-Trinitrobenzene
$C_6H_3N_3O_6$
M.W. = 213.108


Source: M.O.D. Waltham Abbey, England

Batch No. NP/72/78

Date obtained: 14th November 1978

Storage location: Mutagenesis Laboratory - IRI

IRI Notebook reference: 410110/21

Names of Analysts: (i) M.S. Henderson

(ii) J.N. Done

(iii) P. Teale

Analytical Data checked by: (i) J Kilbet (M.S.)

(ii) G A Byrne

Analytical Certificate issued by:

Inveresk Research International
Edinburgh

Date: 28 August 1979

Certificate of Analytical Quality (1,3,5-trinitrobenzene)

TEST	RESULTS AND COMMENTS
Physical appearance	Off-white powder: no detectable odour.
Melting point	**Found:** 119-121°C (uncorrected) **Reported:** 122.5°C (Merck Index)
Solubility data	**Found:** Soluble (10% solution) in acetone, dimethyl sulphoxide, dimethyl formamide, soluble with heating in chloroform, methanol and ethanol. Insoluble in light petroleum, hexane and water.
Elemental analysis	

Element	C	H	N		
Calculated:	33.82	1.42	19.72		
Found (i) :	34.14	1.60	19.32		
(ii):	33.98	1.45	19.12		

Ultraviolet/ visible spectrum

Spectrum attached: 1 mg/100 ml methanol: methanol in reference beam: 10 mm quartz cells: Unicam SP800 spectrophotometer.

λ_{max}, nm	223			
log ε	4.45			

Infrared spectrum

Spectrum attached: KBr disc: Perkin Elmer 257 spectrometer.

Ref: H.F. Shurvell, J.A. Faniran, E.A. Symons & E. Buncel. Canadian J. Chem. 45 117 1967. Good agreement with published figures.

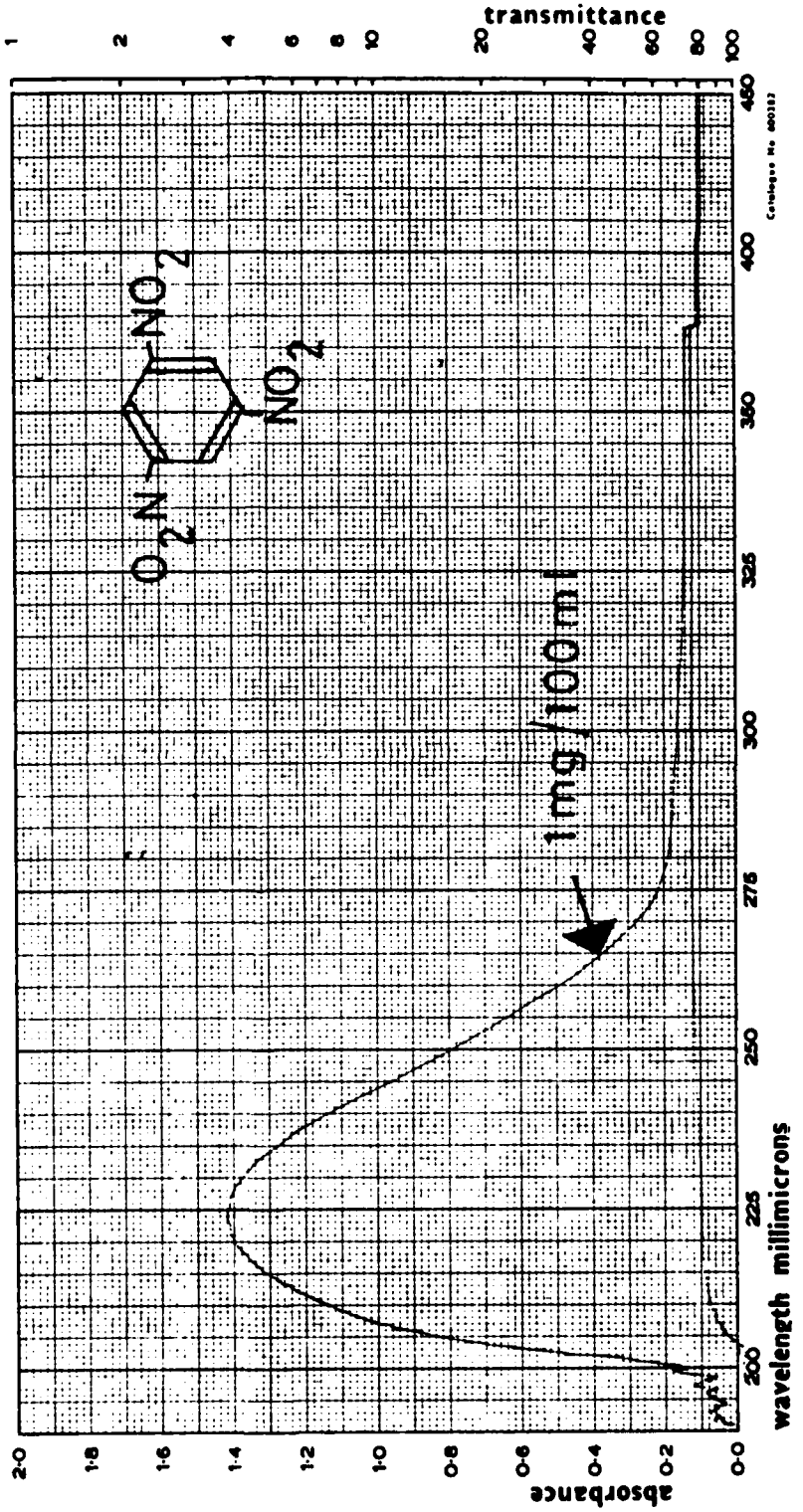
s-$C_6H_3(NO_2)_3$	Assignment
3 100	νCH(νCD) *E'*
1 625	νCC *E'*
1 545	NO_2 asym. stretch *E'*
1 443	νCC *E'*
1 348	NO_2 sym. stretch *E'*
1 075	βCH(βCD) *E'*
918	νCN *E'*
728	NO_2 sym. deform. *E'*
720	γCH(γCD) A_2''
710	φCC A_2''
620	αCCC *E'*
518	NO_2 out-of-plane bend A_2''
360	NO_2 rock *E'*

Certificate of Analytical Quality (1,3,5-trinitrobenzene)

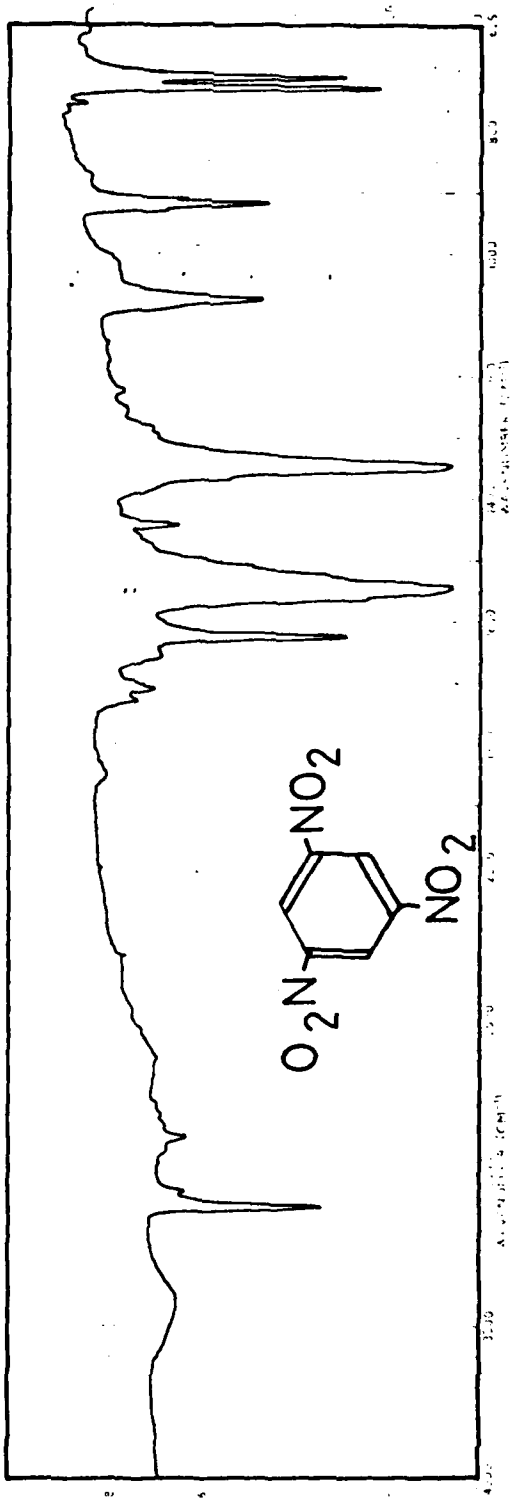
<table>
<tr><td>Proton magnetic resonance spectrum</td><td>Spectrum attached. In d_6 acetone solution on Varian Associates HA-100 spectrometer.

<table><tr><th>δ</th><th>Chemical moiety</th><th>No. protons by integration</th></tr><tr><td>9.36</td><td>Aromatic - H</td><td>-</td></tr></table></td></tr>
<tr><td>Thin-layer chromatography</td><td>Silica gel G (0.25 mm): (i) chloroform (ii) ethylacetate: light petroleum (1:3 v/v) iodine vapour and ultraviolet (254 nm) detection. 500, 1000 & 1500 µg applied.
(i) single spot Rf = 0.51
(ii) single spot Rf = 0.51</td></tr>
<tr><td>High performance liquid chromatography</td><td>Varian 8500 liquid chromatograph
250 x 5 mm Hypersil silica:
i) 1.5% acetonitrile in n-hexane 2 ml/min; U.v. detection 225 nm; chart speed 600 mm/h.
ii) 1% acetonitrile in n-hexane</td></tr>
<tr><td>Gas-liquid chromatography</td><td>-</td></tr>
<tr><td>Mass spectrum</td><td>Finnigan 4000 GC-MS (6110 Data System)
Source 260°C (indicated); EI; 70eV
Mass Spectrum attached.</td></tr>
<tr><td>Other analytical methods</td><td>-</td></tr>
<tr><td>General comments</td><td>i) In order to show the absence of possible impurities, hplc of a mixture of mono-, di- & tri-nitrobenzene was undertaken. Chromatograms attached.
ii) Elemental analyses were performed by Butterworth Laboratories Limited, Teddington, Middlesex.</td></tr>
</table>

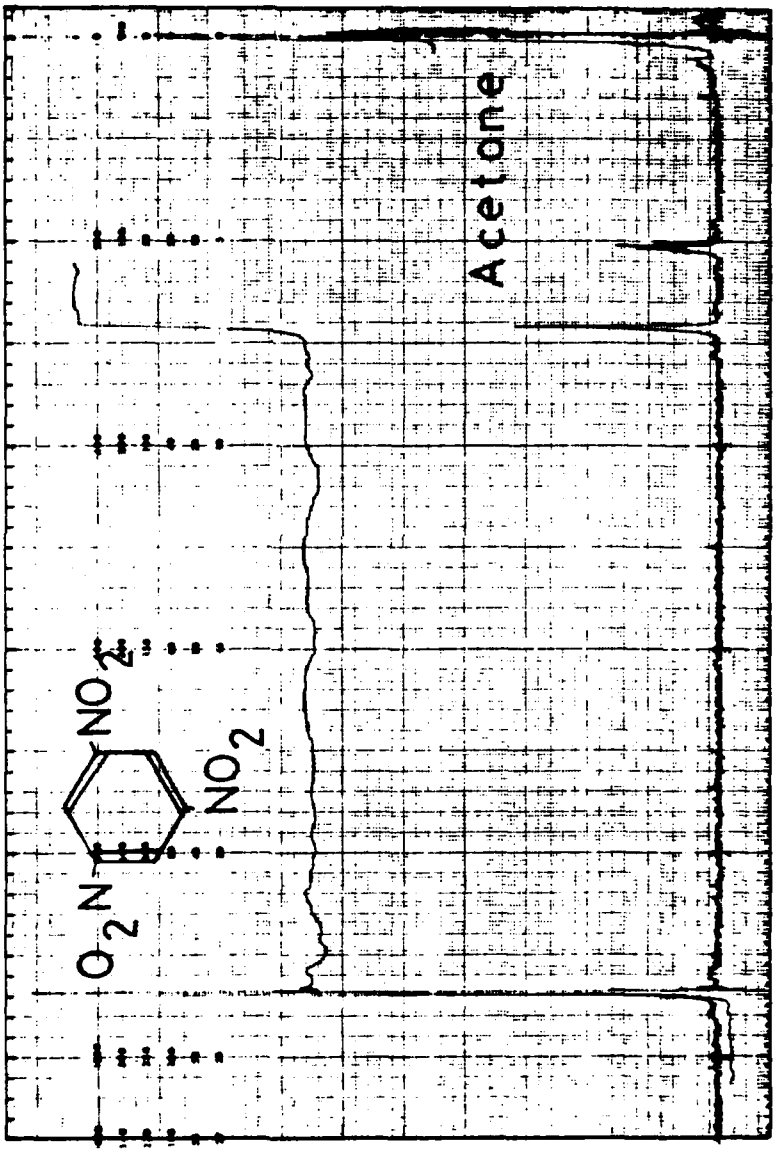
Ultraviolet spectrum of 1,3,5,-trinitrobenzene



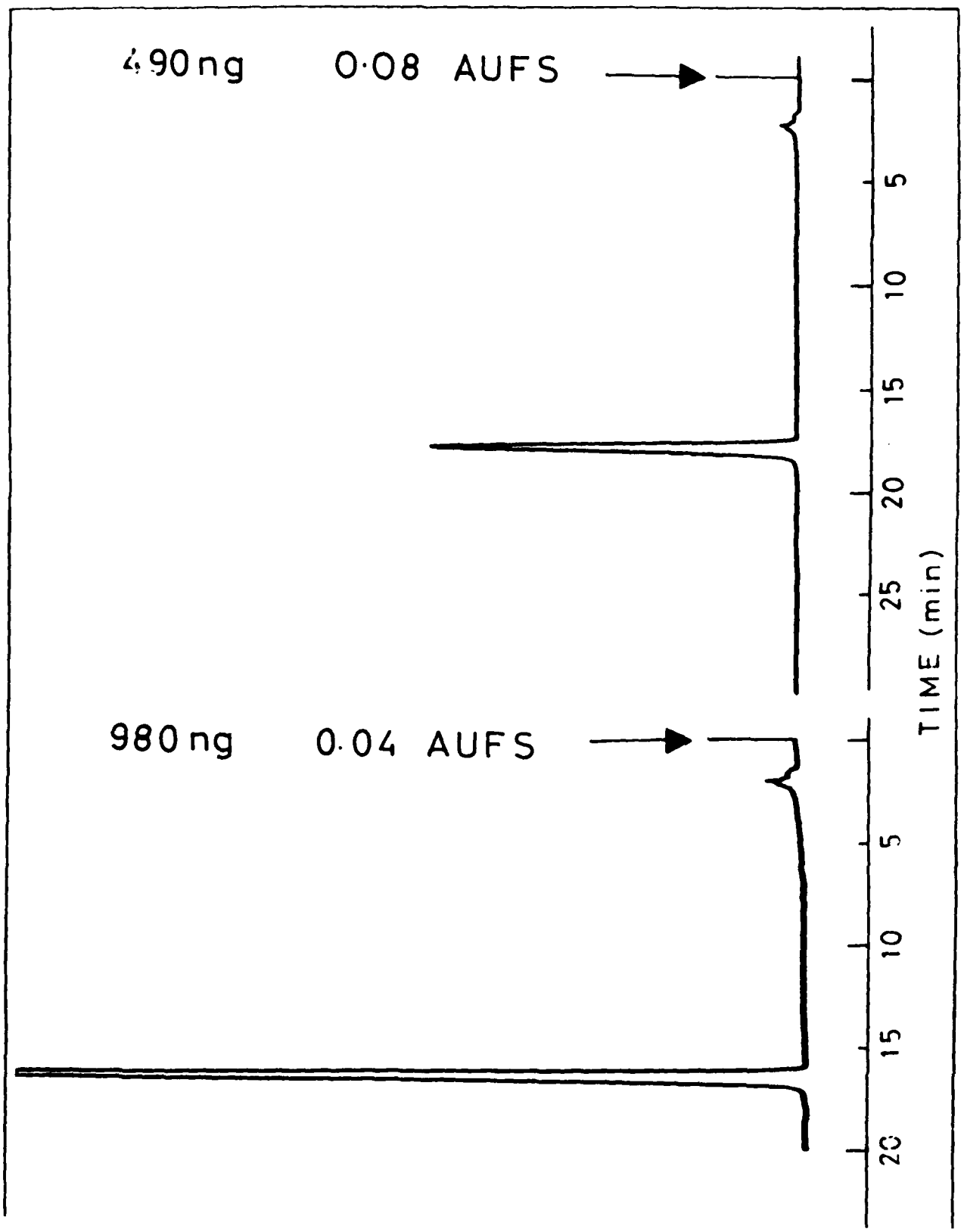
Infrared spectrum of 1,3,5-trinitrobenzene



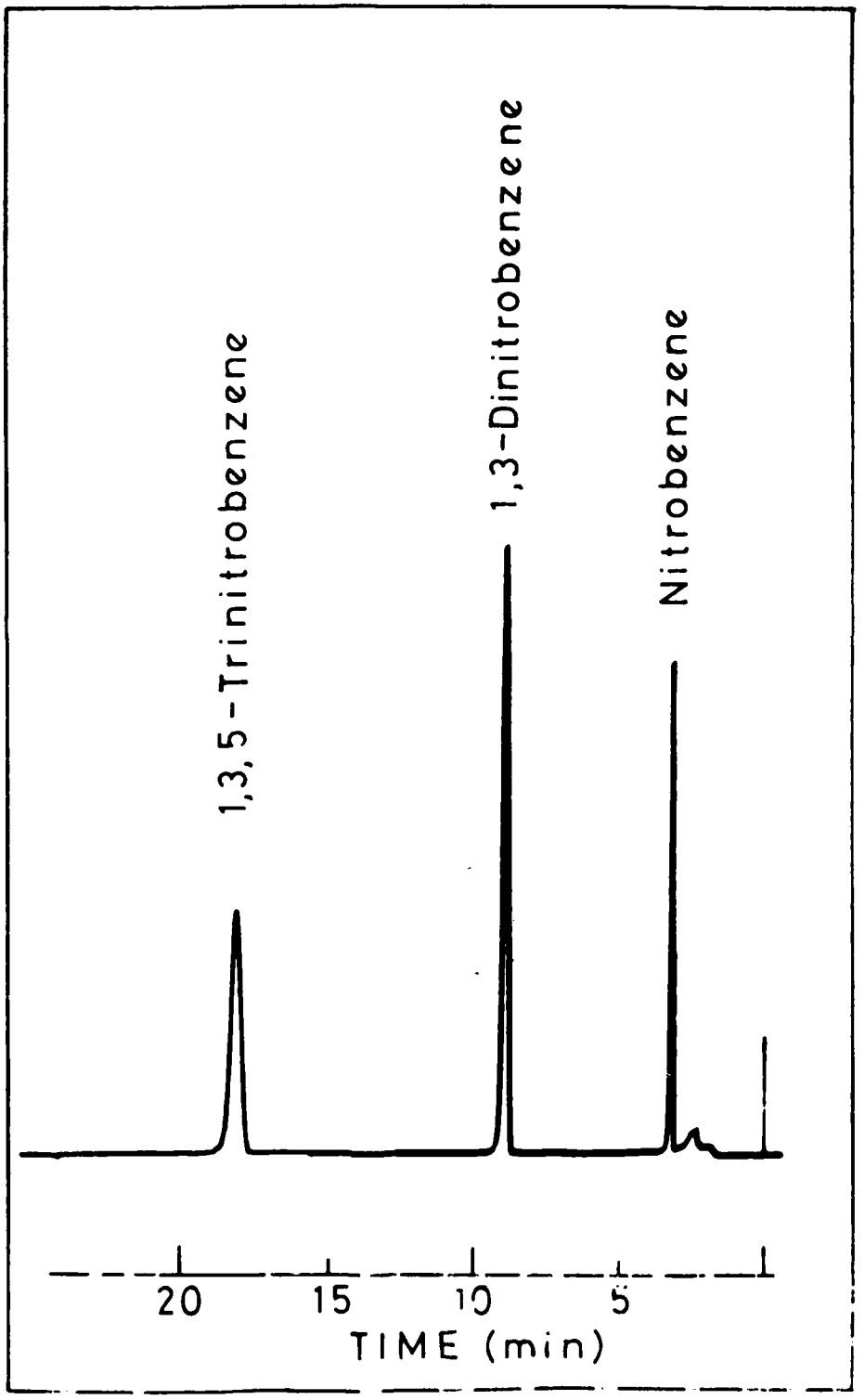
Nuclear magnetic resonance spectrum of 1,3,5,-trinitrobenzene



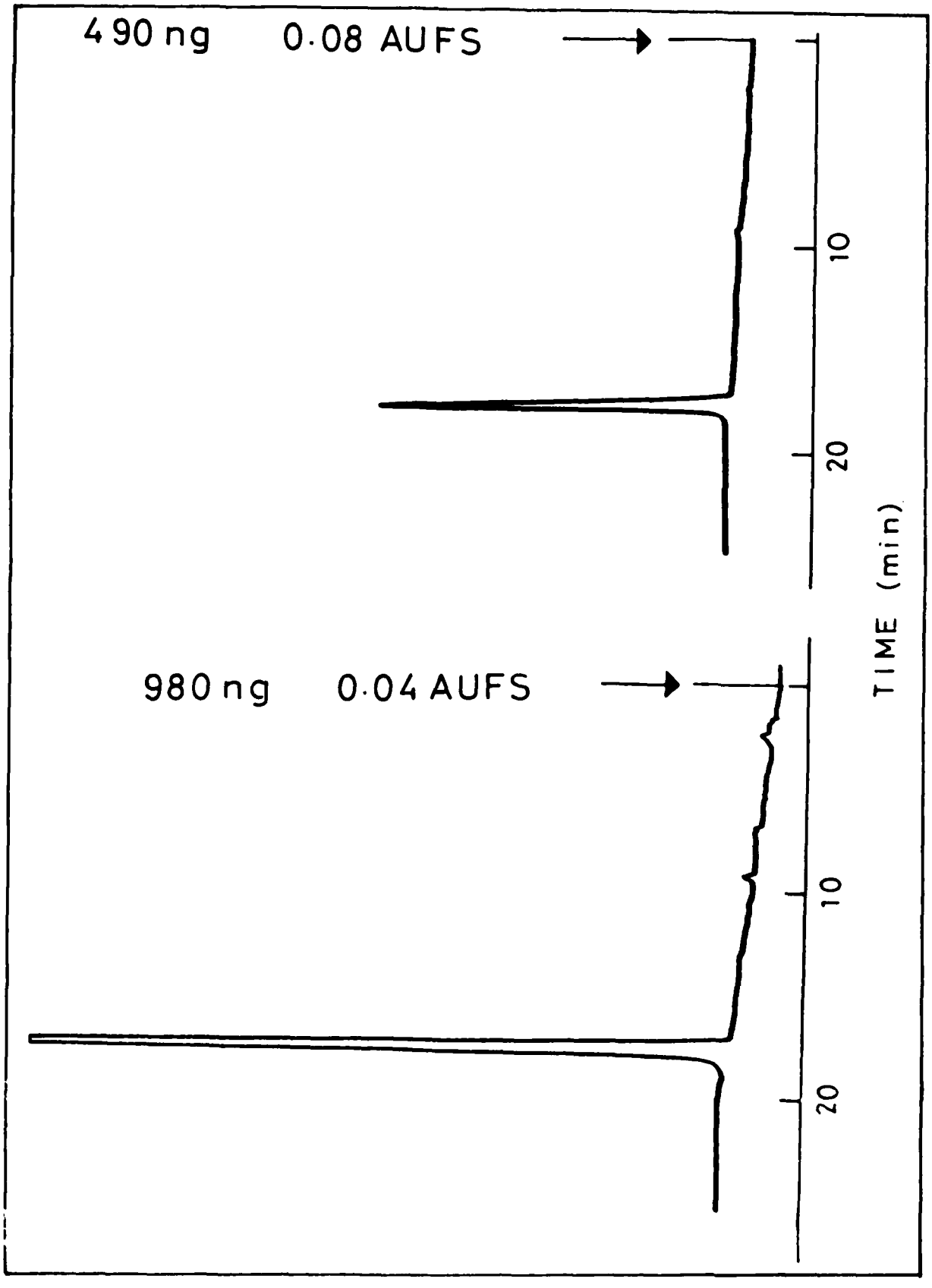
High performance liquid chromatogram of
1,3,5-trinitrobenzene using 1.5% acetonitrile in hexane



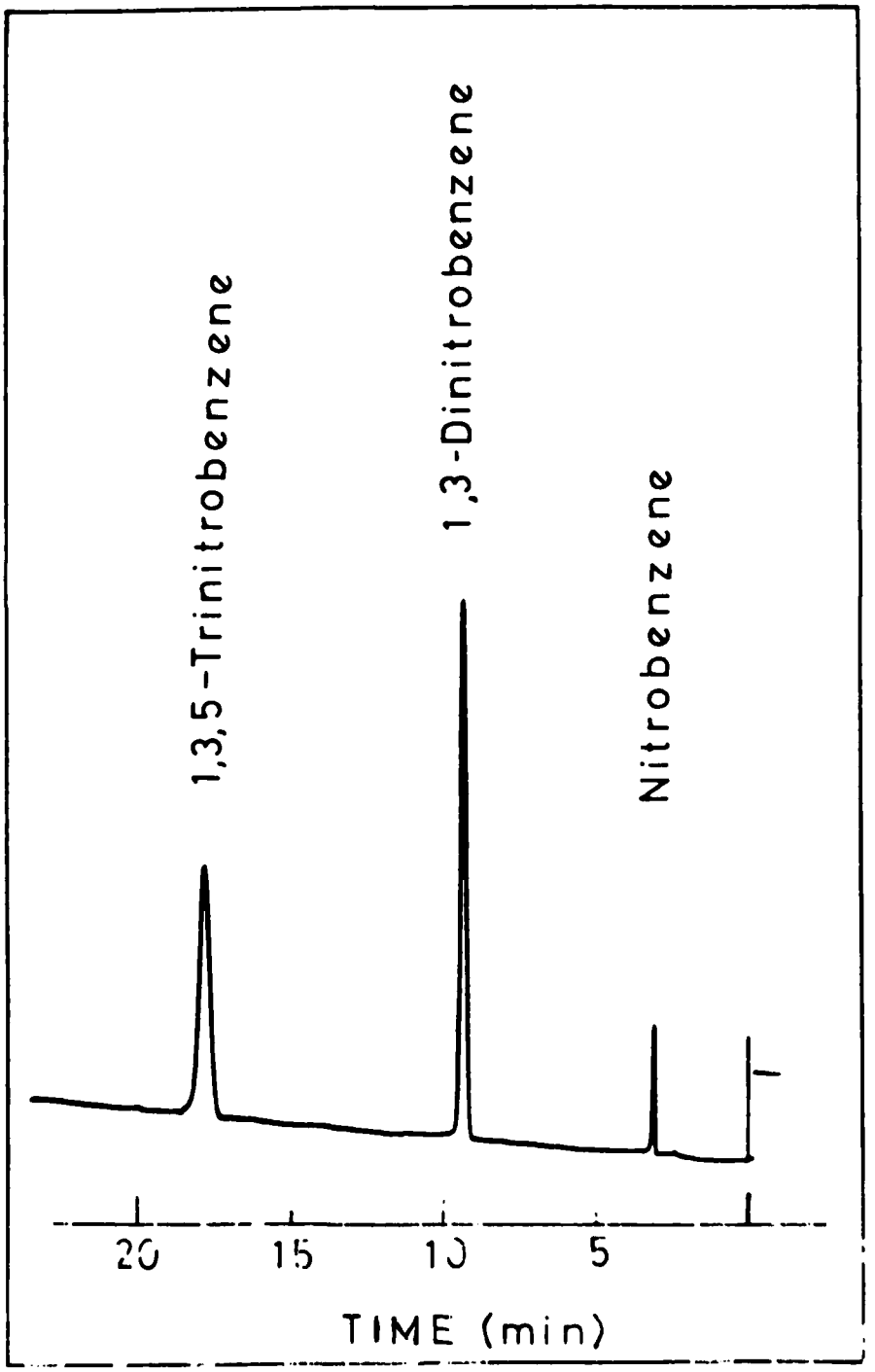
High performance liquid chromatogram of the nitrobenzenes using 1.5% acetonitrile in hexane



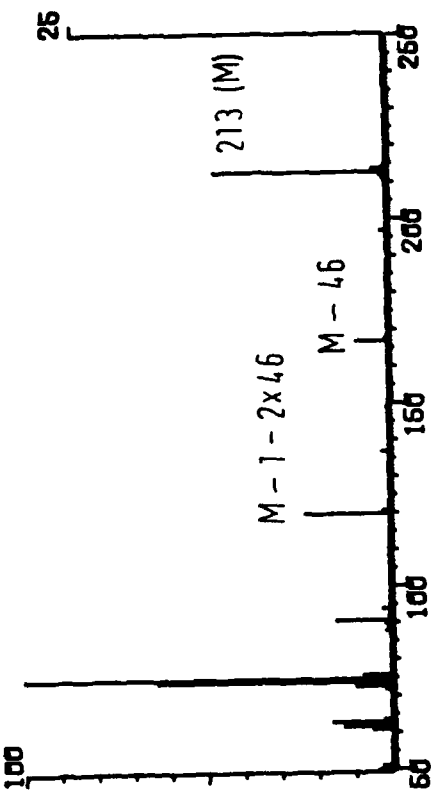
High performance liquid chromatography of
1,3,5-trinitrobenzene using 1% acetonitrile in hexane



High performance liquid chromatogram of the nitrobenzenes in 1% acetonitrile in hexane



Mass spectrum of 1,3,5,-trinitrobenzene



ZINC CHLORIDE

E. coli DNA Repair Test on Plates (Table 144)

Zinc chloride did not show preferential toxicity for the polymerase-deficient strain at an exposure level of 10 mg per plate either in presence or absence of S-9 mix.

S. typhimurium Mutation Tests (Tables 145-147)

A preliminary toxicity test with strain TA 98 showed that zinc chloride was toxic to the bacteria at an exposure level of 3.3 mg per plate.

The mutagenicity tests were conducted with S. typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98 in the presence and absence of S-9 mix (Tables 146 and 147). There was no indication of a mutagenic response, although the compound was toxic at the higher exposure levels, particularly in the absence of S-9 mix.

S. cerevisiae Mitotic Recombinogenic Activity Tests (Tables 148-151)

Tests did not reliably show that zinc chloride was toxic to yeast cells during 150 min incubation. On the other hand, 18 h incubation using incubation method 2 gave a reduced viable count at the lowest concentration tested, 0.75 $mg.ml^{-1}$ (Table 149).

The recombinogenic test conducted in the absence of S-9 mix did not clearly demonstrate that zinc chloride had recombinogenic potential, although recombinant frequency was elevated at 2 concentration levels (18 $mg.ml^{-1}$ and 32 $mg.ml^{-1}$) (Table 150). In the presence of S-9 mix and using incubation method 2, toxicity was severe at 1 $mg.ml^{-1}$ and above. At the 2 low exposure concentrations, toxicity was moderate (50% and 34%), but there was no sign of an increase in recombinant frequency (Table 151).

Conclusions

Genetic damage induction was not demonstrated in the DNA repair test or mutation test with bacteria. Results with the mitotic recombinogenic test were not entirely clear in the absence of S-9 mix, but there was no indication of recombinant induction in the presence of S-9 mix.

REFERENCES

(1) Rauscher, Jr., F.J., (1976), Carcinogenesis Program, Fourth Annual Collaborative Conference.

(2) Di Paulo, J.A., (1973), in "Carcinogenesis Testing of Chemicals", ed. L. Golberg, C.R.C. Press, Cleveland, Ohio, p. 91.

(3) Di Paulo, J.A., Nelson, R.L. and Donovan, P.J., (1973), Arch. Pathol., 95, 380.

(4) Brookes, P. and de Serres, F.J., (1976), Mutation Res., 38, 155.

(5) De Serres, J.F., (1976), Mutation Res., 41, 43.

(6) Gross, J. and Gross, M., (1969), Nature, 224, 1166.

(7) Vogel, H.J. and Bonner, D.M., (1956), J. Biol. Chem., 218, 97.

(8) Slater, E.E., Anderson, M.D. and Rosenkranz, H.S., (1971), Cancer Res., 31, 970.

(9) Longnecker, D.S., Curphey, T.J., James, S.T., Daniel, D.S. and Jacobs, N.J., (1974), Cancer Res., 34, 1638.

(10) Rosenkranz, H.S., Carr, H.S. and Rose, H.M., (1965), J. Bacteriol., 89, 1354.

APPENDIX

Mutagens in the Mitotic Recombinogenic Activity Test

Ethyl methanesulphonate (Table 152)

Ethyl methanesulphonate (EMS), the chosen positive control substance not requiring activation specifically by S-9 mix, was active in the induction of recombinants and total aberrants in a concentration-response related manner. Negative control frequencies were particularly low in this experiment and allowed detection of a significant response at an EMS concentration of 5 $mg.ml^{-1}$. The standard control concentration used in the testing of the 15 munitions compounds was 10 $mg.ml^{-1}$ (i.e. 20 mg per incubation mixture).

Ethyl methanesulphonate with S-9 mix (Tables 153 and 154)

Incubation method 1 was used in these experiments. It has been suggested occasionally that EMS-induced genetic damage may be enhanced by S-9 mix. The possibility of using EMS as a positive control in both the presence and absence of S-9 mix, therefore, was investigated. Three S-9:co-factor solution ratios were tested: i.e. 1:9, 1:3 and 1:1 initially (Table 153). It appeared that the 1:9 and 1:3 ratios might give some enhancement of the recombinogenic effect. This was not confirmed in a second experiment (Table 154).

Aflatoxin B_1 with S-9 mix (Table 155)

Incubation method 1 was used in the experiment. Aflatoxin B_1 at a concentration of 100 $\mu g.ml^{-1}$ (200 µg per incubation mixture) was not a significant recombinogen in this experiment. The highly carcinogenic nature of this compound in any case was a deterrent to its use as a positive control substance.

Dimethylnitrosamine with S-9 mix (Table 153)

Incubation method 1 was used in this experiment. Dimethylnitrosamine (10 $mg.ml^{-1}$) was toxic and there was an increase in recombinant frequency in the presence of S-9 mix at the 1:1 ratio, but this was a consequence of the high toxicity of the compound rather than an increase in recombinant number.

Hydroxylamine with S-9 mix (Table 154)

Incubation method 1 was used in this experiment. Hydroxylamine.HCl (200 $\mu g.ml^{-1}$) was not active in the test either in the presence or absence of S-9 mix.

Cyclophosphamide with S-9 mix (Tables 156-159)

Incubation method 1 was used in experiments reported in Tables 156 and 157 and method 2 was used in experiments reported in Tables 158 and 159.

Cyclophosphamide did not induce any significant increase in recombinant frequency in the method 1 incubations. On the other hand, significant increases were obtained with method 2 incubations. Some increases were seen in the absence of S-9 mix, but there was marked enhancement in the presence of S-9 mix. Whether NADPH or an NADPH-generating mixture is added with the S-9 seemed to be immaterial to the outcome (Table 159).

TABLES

TABLE 1

E. coli: Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 18 June 1979
Date examined: 19 June 1979

Substance: SEX
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): M61041
Numbering colour(s): Blue with S-9
Red with S-9
Culture batch: B

Toxicity	Quantity per Plate	Activation	pol A+ (100 µl)			pol A- (200 µl)		
Dimethylsulphoxide	100 µl	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-
Ethyl methanesulphonate	10 µl	with S-9	19n	18n	18n	28o 18n	27o 17n	26s
		without S-9	19n	19n	18n	26o 18n	26o 18n	26o 18n
Chloramphenicol	30.0 µg	with S-9	30n	29n	30n	28n	29n	29n
		without S-9	31n	32n	31n	30n	29n	29n
SEX	pptn 10.0 mg	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-

Measurement in mm Diameter of hole = 15 mm
o = overall diameter i.e. specific and non-specific killing;
s = specific killing; n = non-specific killing;
pptn = precipitation

TABLE 2

DNA Repair Test in Suspension

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 25 June 1979
Date counted: 26 June 1979

Substance: SEX
Activation: without activation
Liver preparation date: -
Batch no. (plates): M61041
Numbering colour(s): Blue without S-9
Culture batch: C

In Suspension	Quantity per Plate	pol A^+ (100 µl)			pol A^- (100 µl)		
(solvent) Dimethylsulphoxide	100 µl	826	1182	1062	400	407	404
Ethyl methanesulphonate	10 µl	516	455	585	0	0	0
2-Aminofluorene	6.5 µg	885	1082	1111	320	473	311
SEX	10.0 µg	1106	1200	1178	427	363	309
	33.3 µg	1133	1135	1266	489	393	394
	100.0 µg	1083	1149	1104	400	387	309
	333.3 µg	1479	1100	1147	367	296	430
	1.0 mg pptn	702	927	115	294	333	234
	3.3 mg pptn	1050	993	1081	367	371	360
	5.0*mg pptn	993	1123	1225	344	414	368

pptn = precipitation * = saturated solution

TABLE 3

DNA Repair Test in Suspension

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 25 June 1979
Date counted: 26 June 1979

Substance: SEX
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): M61041
Numbering colour(s): Red with S-9
Culture batch: C

In Suspension	Quantity per Plate	pol A^+ (100 µl)			pol A^- (100 µl)		
(solvent) Dimethylsulphoxide	100 µl	1260	1252	1064	755	661	798
Ethyl methanesulphonate	10 µl	1095	1140	1116	1	3	0
2-Aminofluorene	6.5 µg	1218	1238	1428	571	566	419
SEX	10.0 µg	1166	952	1640	671	631	661
	33.3 µg	1484	1509	1584	600	608	549
	100.0 µg	1603	1363	1401	585	695	666
	333.3 µg	1246	1333	1635	688	588	557
	1.0 mg pptn	1405	1322	1363	556	488	721
	3.3 mg pptn	1443	1432	1484	560	606	604
	5.0 mg* pptn	1261	1235	1458	555	647	573

pptn = precipitation * = saturated solution

TABLE 4

Toxicity Test in Strain TA 98

Substance: SEX

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 10 May 1979

Operator(s): Colin Riach

Batch no. (plates): R54140

Numbering colour(s): Red with S-9

Date plated: 4 June 1979

Blue without S-9

Date counted: 6 June 1979

Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	30	29
SEX	10.0 µg	29	17
	33.3 µg	20	17
	100.0 µg	29	21
	333.3 µg	33	17
	1.0 mg	25	14
	3.3 mg	29	9
	10.0 mg pptn	35	20

pptn = precipitation

TABLE 5

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: SEX

Project no: 410110
Activation: Aroclor-induced Fischer rat
Contractor: US Army
Liver preparation date: 18 May 1979
Operator(s): Colin Riach
Batch no. (plates): M35044
Jennifer Harvey
Numbering colour(s): Red with S-9
Date plated: 6 June 1979
Blue without S-9
Date counted: 8 June 1979
Culture batch: A

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	6 8 3	7 6 8	25 22 23	15 11 9
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	24 33 22	17 20 31	292 208 257	167 168 194
SEX	10.0 µg	7 8 6	9 5 4	27 13 24	15 18 13
	33.3 µg	6 4 8	9 5 4	17 16 19	12 11 9
	100.0 µg	7 7 8	12 7 6	17 21 20	5 10 11
	333.3 µg	8 6 7	9 5 3	25 15 22	9 9 13
	1.0 mg	6 6 7	4 6 7	22 24 29	8 10 14
	3.3 mg	6 6 5	5 7 7	20 17 24	12 18 20
	pptn 10.0 mg	4 8 5	10 5 10	40 20 33	12 14 9

pptn = precipitation

TABLE 6

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: SEX

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Colin Riach

Batch no. (plates): M43241

Jennifer Harvey

Numbering colour(s): Red with S-9

Date plated: 18 June 1979

Blue without S-9

Date counted: 20 June 1979

Culture batch: B

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	13 12 3	8 13 7	21 20 14	15 16 14	108 91 102	84 74 75
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	33 36 31	3044 2862 2724	650 872 659	283 333 346	659 594 780	283 218 215
SEX	10.0 µg	14 4 7	8 12 6	25 30 15	9 17 14	116 78 80	76 71 86
	33.3 µg	17 3 7	6 3 8	20 24 25	16 16 18	104 96 116	65 91 76
	100.0 µg	12 6 17	4 3 8	29 19 33	17 18 16	93 100 96	85 80 92
	333.3 µg	13 6 8	8 13 6	19 15 27	22 9 12	97 81 89	87 98 99
	1.0 mg	8 7 6	7 8 4	33 21 17	13 15 17	134 102 102	85 64 84
	3.3 mg	4 13 7	6 12 8	25 25 33	18 15 8	97 100 134	87 134 98
	10.0 mg pptn	9 4 7	5 4 3	29 32 29	10 10 18	90 96 102	103 97 97

pptn = precipitation

TABLE 7

Saccharomyces cerevisiae D5 Toxicity Test
Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 7 June 1979
Date counted: 11 June 1979

Substance: SEX
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 May 1979
Batch no. (plates) M53150
Numbering colour(s): Black with S-9
Blue without S-9

1. With activation

Incubation Time		30 min	60 min	90 min	120 min
Substance	Quantity				
Dimethylsulphoxide	200 µl	330	281	290	250
SEX	2.0 mg	304	265	276	214
	10.0 mg	297	280	245	225
	20.0 mg	316	283	293	234

Conclusion: Dose range: 20 mg, 10 mg, 5 mg, 2.5 mg, 6.25 µg, 312.5 µg Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min
Substance	Quantity				
Dimethylsulphoxide	200 µl	272	276	294	272
SEX	2.0 mg	253	290	277	240
	10.0 mg	229	288	282	265
	20.0 mg	241	242	243	269

Conclusion: Dose range: 20 mg, 10 mg, 5 mg, 2.5 mg, 1.25 mg, 6.25 µg, 312.5 µg Incubation time: 2 h

Note: 20 mg i.e. 100 mg/ml solution = saturated solution

TABLE 8

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: SEX

Project no: 410110
Activation: Aroclor-induced Fischer rat
Contractor: US Army
Liver preparation date: 18 June 1979
Operator(s): Rowan Hastwell
Batch no. (plates): BO1441
Jennifer Harvey
Numbering System : β
Date plated: 17 July 1979
Date counted: 23 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	1500	5×10^4	7.5×10^7/ml
SEX	$\beta 3_{250}$ µg	1434	5×10^4	7.2×10^7/ml
	$\beta 2_{2.5}$ mg	1501	5×10^4	7.5×10^7/ml
	$\beta 1_{10}$ mg pptn	1466	5×10^4	7.3×10^7/ml

pptn = precipitation

TABLE 8 (continued)

Conclusion:-	Doses for full test	Dilution factor
β7	156.25 µg	5×10^4
β6	312.5 µg	5×10^4
β5	625 µg	5×10^4
β4	1.25 mg	5×10^4
β3	2.5 mg	5×10^4
β2	5 mg	5×10^4
β1	10 mg	5×10^4

TABLE 9

Saccharomyces cerevisiae D5 Recombination, without activation

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	SEX
Incubation time:	2 h
Date plated:	12 June 1979
Date counted:	22 June 1979
Plates (batch):	M53150

TABLE 9 (continued)

Saccharomyces cerevisiae D5 Recombination, without activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	14,276	100	0	0	2.8 (4)	0.7 (1)	2.8 (4)	1.4 (2)	2.1 (3)	0	9.8 (14)
EMS	Dose 8 +ve control 20 mg 80.5mM	21,383	149.8	56.6 *(121)*	22.0 *(47)*	11.7 *(25)*	7.0 *(15)*	87.9 *(188)*	74.8 *(160)*	98.7 *(211)*	78.6 *(168)*	358.7 *(767)*
SEX	Dose 7 312.5 µg	18,003	126.1	0.6 (1)	0.6 (1)	0.6 (1)	0.6 (1)	1.7 (3)	0.6 (1)	1.1 (2)	1.1 (2)	5.6 (10)
	Dose 6 625 µg	8,240	57.7	2.4 (2)	0	0	3.6 (3)	2.4 (2)	1.2 (1)	0	2.4 (2)	9.7 (8)
	Dose 5 1250 µg	20,797	145.7 pptn	1.0 (2)	0.5 (1)	0	0.5 (1)	1.5 (3)	1.5 (3)	0.5 (1)	1.5 (3)	5.3 (11)
	Dose 4 2.5 mg	19,701	138.0 pptn	0	0	0	1.0 (2)	0.5 (1)	0.5 (1)	0.5 (1)	0	2.5 (5)
	Dose 3 5 mg	19,345	135.5 pptn	0.5 (1)	0	0.5 (1)	1.0 (2)	0	1.6 (3)	0	0.5 (1)	3.6 (7)
	Dose 2 10 mg	19,715	138.1 pptn	0	0	0.5 (1)	0.5 (1)	1.0 (2)	1.0 (2)	0	0	3.0 (6)
	Dose 1 20 mg	18,086	126.7 pptn	0.6 (1)	0	1.1 (2)	0.6 (1)	1.7 (3)	0.6 (1)	0.6 (1)	0.6 (1)	5.0 (9)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 10

S. cerevisiae D5 Recombinogenic Activity
with SEX, with Metabolic Activation,
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Jennifer Harvey
	Colin Riach
Substance:	SEX
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer Rat
Liver preparation date:	18 July 1979
Date plated:	25 July 1979
Date counted	3 August 1979
Plates (Batch)	BO1441

Dilution Factors used to dilute incubation tubes
after 18 h incubation

Substance	Dose		Dilution Factor
Dimethylsulphoxide	100	µl	2.7×10^4
Cyclophosphamide	38.9	mg	3.3×10^4
SEX	156	µg	2.7×10^4
SEX	313	µg	2.7×10^4
SEX	625	µg	2.7×10^4
SEX	125	µg	2.7×10^4
SEX	2.5	mg	2.7×10^4
SEX	5	mg	2.7×10^4
SEX	10	mg	2.7×10^4

TABLE 10 (continued)

S. cerevisiae D5 *Recombinogenic* Activity

with SEX, with Metabolic Activation

Substance	Dose	Colonies Counted: Viable Count per ml after 18 h incubation	% survival	No. of aberrants/10^4 survivors: Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline	Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	21,145 / 5.7×10^7	100	0.5 (1)	0	0.9 (2)	0.9 (2)	1.9 (4)	0	2.4 (5)	0.5 (1)	6.6 (14)
Cyclo-phos-phamide	Dose 8 +ve control 38.9 mg 70 mM	27,371 / 9.0×10^7	158	1.5 (4)	0.7 (2)	4.4 (12)	0.7 (2)	4.0 (11)	0	0.7 (2)	2.2 (6)	12.1 (33)
SEX	Dose 7 156 µg	20,861 / 5.6×10^7	98	0	0	1.0 (2)	0	2.4 (5)	0.5 (1)	1.9 (4)	0	5.8 (12)
	Dose 6 313 µg	20,957 / 5.7×10^7	100	0.5 (1)	1.0 (2)	1.4 (3)	1.0 (2)	1.4 (3)	0	1.4 (3)	1.4 (3)	6.7 (14)
	Dose 5 625 µg	21,276 / 5.7×10^7	100	0	0	0.9 (2)	0.5 (1)	2.8 (6)	0.5 (1)	1.9 (4)	0	6.6 (14)
	Dose 4 1250 µg	25,736 / 6.9×10^7	121	0	0.4 (1)	0.8 (2)	0	0.8 (2)	0.4 (1)	0	0.4 (1)	2.3 (6)
	Dose 3 2.5 mg	21,127 / 5.7×10^7	100	0	0	1.4 (3)	0.9 (2)	0.9 (2)	1.4 (3)	0	0	4.7 (10)
	Dose 2 5 mg	26,152 / 7.1×10^7	125	0.4 (1)	0	1.9 (5)	0.4 (1)	0.8 (2)	0.8 (2)	1.1 (3)	0.4 (1)	5.4 (14)
	Dose 1 10 mg	26,594 / 7.2×10^7	126	0.4 (1)	0	0.4 (1)	1.1 (3)	0.8 (2)	0.4 (1)	1.1 (3)	0.4 (1)	4.1 (11)

Figures in parentheses are actual number of aberrants counted

TABLE 11

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 18 June 1979
Date examined: 19 June 1979

Substance: TAX
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): M61041
Numbering colour(s): Blue with S-9
Red without S-9
Culture batch: B

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
TAX	10.0 mg pptn	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-

For controls see Table 1.
Measurements in mm. Diameter of hole = 15 mm
pptn = precipitation

AD-A084 121 INVERESK RESEARCH INTERNATIONAL LTD EDINBURGH (SCOTLAND) F/G 6/5
MUTAGENICITY AND DNA REPAIR POTENTIAL OF 15 CHEMICALS.(U)
JAN 80 D B MCGREGOR DAMD17-78-C-8064

UNCLASSIFIED NL

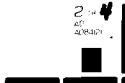


TABLE 12

DNA Repair Test in Suspension

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 25 June 1979
Date counted: 26 June 1979

Substance: TAX
Activation: without activation
Liver preparation date: -
Batch no. (plates): M61041
Numbering colour(s): Blue without S-9
Culture batch: C

In Suspension	Quantity per Plate	pol A^+ (100 µl)			pol A^- (100 µl)		
(solvent) Dimethylsulphoxide	100 µl	826	1182	1062	400	407	404
Ethyl methanesulphonate	10 µl	516	455	585	0	0	0
2-Aminofluorene	6.5 µg	885	1082	1111	320	473	311
TAX	10.0 µg	870	964	1013	274	400	362
	33.3 µg	858	1101	1084	428	416	309
	100.0 µg	798	865	993	322	437	362
	333.3 µg	889	987	927	333	401	361
	1.0 mg	919	1040	1109	245	264	375
	3.3 mg	969	1037	916	284	150	313
	8.1 mg* pptn	841	1032	1122	374	498	363

pptn = precipitation * = saturated solution

TABLE 13

DNA Repair Test in Suspension

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 25 June 1979
Date counted: 26 June 1979

Substance: TAX
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): M61041
Numbering colour(s): Red with S-9
Culture batch: C

In suspension	Quantity per Plate	pol A^+ (100 µl)			pol A^- (100 µl)		
(solvent) Dimethylsulphoxide	100 µl	1260	1252	1064	755	661	798
Ethyl methanesulphonate	10 µl	1095	1140	1116	1	3	0
2-Aminofluorene	6.5 µg	1218	1238	1428	571	566	419
TAX	10.0 µg	1315	1089	1386	596	685	661
	33.3 µg	1383	1369	1374	518	630	672
	100.0 µg	1500	1189	1289	670	589	590
	333.3 µg	1147	1386	1560	455	518	647
	1.0 mg	1401	1426	1215	541	427	589
	3.3 mg	1085	1190	1033	529	553	616
	8.1 mg* pptn	1407	1096	1176	552	629	565

pptn = precipitation * = saturated solution

TABLE 14

Toxicity Test in Strain TA 98

Substance: TAX

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 18 May 1979

Operator(s): Colin Riach
Batch no. (plates): R54140

Numbering colour(s): Red with S-9

Date plated: 4 June 1979
Blue without S-9

Date counted: 6 June 1979
Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	27	8
TAX	10.0 µg	32	14
	33.3 µg	36	15
	100.0 µg	37	15
	333.3 µg	26	13
	1.0 mg	31	13
	3.3 mg	28	21
	10.0 mg	19	27

TABLE 15

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: TAX

Project no: 410110
Activation: Aroclor-induced Fischer rat
Contractor: US Army
Liver preparation date: 18 May 1979
Operator(s): Colin Riach
Batch no. (plates): M35044
Jennifer Harvey
Numbering colour(s): Red with S-9
Date plated: 6 June 1979
Blue without S-9
Date counted: 8 June 1979
Culture batch: A

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	6 8 3	7 6 8	25 22 23	15 11 9
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	24 33 22	17 20 31	292 208 257	167 168 194
TAX	10.0 µg	5 11 10	5 6 6	24 16 19	10 12 13
	33.3 µg	3 6 7	3 10 11	21 18 34	12 7 11
	100.0 µg	7 7 7	7 6 8	18 25 17	15 12 14
	333.3 µg	4 5 6	10 7 11	22 24 15	15 7 17
	1.0 mg	3 8 6	6 3 8	18 12 18	17 9 8
	3.3 mg	6 4 3	6 7 3	10 18 11	11 8 10
	10.0 mg	4 3 5	7 9 11	17 24 23	5 8 9

TABLE 16

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: TAX

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 18 June 1979

Operator(s): Colin Riach
Batch no. (plates): M43241

Jennifer Harvey
Numbering colour(s): Red with S-9

Date plated: 18 June 1979
Blue without S-9

Date counted: 20 June 1979
Culture batch: B

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	13 12 3	8 13 7	21 20 14	15 16 14	108 91 102	84 74 75
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	33 36 31	3044 2862 2724	650 872 659	283 333 346	659 594 780	283 218 215
TAX	10.0 µg	5 8 9	2 8 5	13 12 19	17 22 17	93 108 80	88 96 97
	33.3 µg	9 8 7	15 8 5	18 19 16	14 14 16	112 98 100	92 86 75
	100.0 µg	9 7 4	7 2 2	15 21 29	18 16 16	98 96 99	76 89 99
	333.3 µg	9 3 8	8 8 12	13 15 12	19 25 16	108 91 104	85 92 97
	1.0 mg	4 2 9	2 5 13	29 21 25	16 17 15	99 100 132	108 109 79
	3.3 mg	3 5 7	5 6 9	17 17 24	12 18 22	84 101 97	79 86 81
	10.0 mg	7 19 6	3 13 13	19 33 28	9 22 19	102 109 102	99 101 77

TABLE 17

Saccharomyces cerevisiae D5 Toxicity Test
Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Jennifer Harvey
Date plated: 6 June 1979
Date counted: 11 June 1979

Substance: TAX
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 May 1979
Batch no. (plates) M53150
Numbering colour(s): Blue without S-9
Black with S-9

1. With activation

Incubation Time		30 min	60 min	90 min	120 min
Substance	Quantity				
Dimethylsulphoxide	200 µl	330	281	290	250
TAX	2.0 mg	221	258	294	232
	15.15 mg	312	296	241	272
	30.3 mg	273	301	290	271

Conclusion: Dose range: Sat, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg, 500 µg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min
Substance	Quantity				
Dimethylsulphoxide	200 µl	272	276	294	272
TAX	2.0 mg	266	259	300	267
	15.15 mg	274	247	262	250
	30.3 mg	283	270	296	267

Conclusion: Dose range: Sat, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg, 500 µg
Incubation time: 2 h

TABLE 18

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: TAX

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System: Δ

Date plated: 17 July 1979

Date counted: 23 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	1500	5×10^4	7.5×10^7/ml
TAX	$\Delta^3$250 µg	1337	5×10^4	6.7×10^7/ml
	$\Delta^2$2.5 mg	1638	5×10^4	8.2×10^7/ml
	$\Delta^1$10 mg	1364	5×10^4	6.8×10^7/ml

TABLE 18 (continued)

Conclusion:-	Doses for full test	Dilution factor
	Δ7 156.25 µg	5×10^4
	Δ6 312.5 µg	5×10^4
	Δ5 625 µg	5×10^4
	Δ4 1.25 mg	5×10^4
	Δ3 2.5 mg	5×10^4
	Δ2 5 mg	5×10^4
	Δ1 10 mg	5×10^4

TABLE 19

Saccharomyces cerevisiae D5 Recombination
without activation

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	TAX
Incubation time:	2 h
Date plated:	19 June 1979
Date counted:	29 June 1979
Plates (batch):	B04040

TABLE 19 (continued)

Saccharomyces cerevisiae D5 Recombination, without activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	21,026	100.0	0	0.5 (1)	1.0 (2)	0.5 (1)	0.5 (1)	1.4 (3)	0	0.5 (1)	3.8 (8)
EMS	Dose 8 +ve control 20 mg 80.5mM	23,683	112.6	29.1 (69)	14.8 (35)	8.0 (19)	4.6 (11)	73.5 (174)	46.4 (110)	92.0 (218)	43.9 (104)	268.5 (636)
TAX	Dose 7 500.0µg	21,998	104.6	0	0	0.5 (1)	0.5 (1)	0.9 (2)	0.5 (1)	0.5 (1)	0	2.7 (6)
	Dose 6 1.0 mg	21,843	103.9	0.9 (2)	0	1.4 (3)	0.5 (1)	1.4 (3)	0.9 (2)	0.5 (1)	0.9 (2)	5.5 (12)
	Dose 5 2.0 mg	23,802	113.2	0	0.4 (1)	0	1.7 (4)	0.8 (2)	0.8 (2)	0.8 (2)	0.4 (1)	4.6 (11)
	Dose 4 4.0 mg	21,812	103.7	0	0.5 (1)	0	0.5 (1)	0.5 (1)	0.5 (1)	0.5 (1)	0.5 (1)	2.3 (5)
	Dose 3 8.0 mg	22,115	105.2	0	0	0.5 (1)	0.9 (2)	0.5 (1)	0.5 (1)	0.5 (1)	0	2.7 (6)
	Dose 2 16.0 mg	22,516	107.1 pptn	0	0	2.2 (5)	0.4 (1)	0.9 (2)	1.3 (3)	0.9 (2)	0	5.8 (13)
	Dose 1 36.1 mg	24,369	115.9 pptn	0.4 (1)	0	0	0.8 (2)	0.4 (1)	0.8 (2)	0.4 (1)	0.4 (1)	2.9 (7)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide

EMS = Ethyl methanesulphonate

TABLE 20

S. cerevisiae D5 Recombinogenic Activity
with TAX, with Metabolic Activation,
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Jennifer Harvey
	Colin Riach
Substance:	TAX
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer Rat
Liver preparation date:	18 July 1979
Date plated:	31 July 1979
Date counted:	10 August 1979
Plates (Batch)	R16040

Dilution Factors used to dilute incubation tubes
after 18 h incubation

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	2.6×10^4
Cyclophosphamide	41.6 mg	5×10^4
TAX	156 µg	2.6×10^4
TAX	312 µg	2.6×10^4
TAX	625 µg	2.6×10^4
TAX	1.3 mg	2.6×10^4
TAX	2.5 mg	2.6×10^4
TAX	5 mg	2.6×10^4
TAX	10 mg	2.6×10^4

TABLE 20 (continued)

S. cerevisiae D5 Recombinogenic Activity
with TAX, with Metabolic Activation,
Modified Incubation

Substance	Dose	Colonies Counted: Viable count per ml after 18 h incubation	% survival	No. of aberrants/10^4 survivors: Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline	Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
Dimethyl-Sulphoxide	Dose 9 -ve control 100 µl	20,896 / 5.4×10^7	100	0.5 (1)	0	1.9 (4)	0.5 (1)	0.5 (1)	1.0 (2)	1.0 (2)	0.5 (1)	5.3 (11)
Cyclo-phos-phamide	Dose 8 +ve control 41.6 mg 74 mM	18,631 / 9.3×10^7	172	2.1 (4)	2.7 (5)	2.1 (4)	0.5 (1)	3.2 (6)	1.1 (2)	0.5 (1)	4.8 (9)	12.3 (23)
TAX	Dose 7 156 µg	19,430 / 5.1×10^7	94	0.5 (1)	0	1.0 (2)	0	3.1 (6)	0.5 (1)	3.6 (2)	0.5 (1)	8.7 (17)
	Dose 6 312 µg	18,481 / 4.8×10^7	89	0	1.6 (3)	1.1 (2)	0.5 (1)	1.1 (2)	0	0.5 (1)	1.6 (3)	4.9 (9)
	Dose 5 625 µg	19,692 / 5.1×10^7	94	0.5 (1)	0.5 (1)	1.0 (2)	1.0 (2)	0.5 (1)	2.0 (4)	0.5 (1)	1.0 (2)	6.1 (12)
	Dose 4 1.3 mg	20,684 / 5.4×10^7	100	0	1.0 (2)	1.0 (2)	0	0.5 (1)	1.5 (3)	1.9 (4)	1.0 (2)	5.8 (12)
	Dose 3 2.5 mg	20,704 / 5.4×10^7	100	1.4 (3)	0	0.5 (1)	1.0 (2)	0.5 (1)	1.4 (3)	1.0 (2)	1.4 (3)	5.8 (12)
	Dose 2 5 mg	25,105 / 6.5×10^7	120	0	0	0.8 (2)	0.8 (2)	0.8 (2)	0.4 (1)	0.8 (2)	0	6.0 (9)
	Dose 1 10 mg	26,345 / 6.8×10^7	126	0	0.4 (1)	0.4 (1)	0	2.3 (6)	1.5 (4)	1.5 (4)	0.4 (1)	6.1 (16)

Figures in parentheses are actual number of aberrants counted

TABLE 21

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Ethyl centralite
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Dimethylsulphoxide	100 µl	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-
Ethyl *methanesulphonate*	10 µl	with S-9	17n	16n	17n	22s	22s	22s
		without S-9	18n	20n	20n	20s	24s	22s
Chloramphenicol	30.0 µg	with S-9	35n	34n	36n	31n	34n	34n
		without S-9	35n	36n	36n	34n	34n	35n
Ethyl centralite	10.0 mg pptn	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-

pptn = precipitation
n = non-specific killing i.e. complete killing
s = specific killing i.e. selective killing

Diameter of clearing in mm
Diameter of hole = 15 mm

TABLE 22

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 31 January 1979
Date examined: 1 February 1979

Substance: Ethyl centralite
Activation: -
Liver preparation date: -
Batch no. (plates): P30141
Numbering colour Blue

In Suspension	Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide	100 µl	997	1016	1156	1141	1103	1162
Ethyl methanesulphonate	10 µl	593	445	545	0	0	0
2-Aminofluorene	6.5 µg	1048	1013	1040	1030	1043	966
Tube I.D.	Quantity						
G	10.0 µg	845	1012	916	936	419	1082
F	33.3 µg	933	993	1069	1125	1008	966
E	100.0 µg	912	1112	929	1030	1095	867
D	333.3 µg	880	1100	962	925	968	1074
C	1.0 mg pptn	913	1079	830	1025	1029	1033
B	3.3 mg pptn	878	1093	925	971	1073	921
A	10.0 mg pptn	1066	927	950	1148	1098	1116

pptn = precipitation

TABLE 23

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 31 January 1979
Date examined: 1 February 1979

Substance: Ethyl centralite
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour Red

In Suspension		Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide		100 µl	1071	1371	1521	1147	1138	1260
Ethyl methanesulphonate		10 µl	1059	1101	1035	5	7	2
2-Aminofluorene		6.5 µg	1344	1474	1445	1256	1071	1240
Tube I.D.	Quantity							
G	10.0 µg		1366	1157	1426	843	1195	952
F	33.3 µg		1457	1501	1757	1123	1184	1263
E	100.0 µg		1405	1485	1281	1183	1196	1223
D	333.3 µg		1370	1515	1332	1143	1244	1329
C	1.0 mg pptn		1194	1171	1371	1004	1072	1126
B	3.3 mg pptn		1450	1273	1173	989	1106	1051
A	10.0 mg*							

pptn = precipitation
* = precipitate too dense to enable accurate count

TABLE 24

Toxicity Test in Strain TA 98

Substance: Ethyl centralite

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 25 May 1978

Operator(s): Rowan Hastwell

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 4 December 1978

Blue = without S-9

Date counted: 6 December 1978

Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
Ethyl centralite	10.0 µg	28	18
	33.3 µg	34	18
	100.0 µg	30	18
	333.3 µg	25	21
	1.0 mg	22	17
	3.3 mg pptn	31	19
	10.0 mg*		

pptn = precipitation
*precipitation too dense to enable accurate counting

TABLE 25

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Ethyl centralite

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 19 December 1978

Blue = without S-9

Date counted: 21 December 1978

Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	16 9 15	8 18 8	28 25 17	24 17 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	15 18 22	149 145 170	92 114 187	307 275 283
Ethyl centralite	3.3 µg	22 11 12	18 29 19	25 20 24	16 16 17
	10.0 µg	15 9 11	18 16 18	23 14 26	16 20 25
	33.3 µg	21 17 20	10 19 20	20 18 27	19 30 20
	100.0 µg	18 16 12	18 13 8	16 21 19	21 20 16
	333.3 µg	13 20 12	21 17 15	15 21 18	18 23 25
	1.0 mg pptn	12 8 20	16 21 17	20 15 25	21 19 19
	3.3 mg pptn	10 16 16	18TL 10TL 12TL	20 18 22	29STL 18STL 27STL

pptn = precipitation: STL = slightly thin lawn: TL = thin lawn

TABLE 26

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: Ethyl centralite

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): P67542

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 9 January 1979

Blue = without S-9

Date counted: 11 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	5 11 2	7 2 2	18 17 16	16 22 19	112 122 90	102 99 86
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	25 18 20	423 394 470	120 77 104	347 382 406	223 180 203	268 255 274
Ethyl centralite	3.3 µg	5 9 11	3 6 9	18 20 contam	18 25 17	105 119 129	123 116 141
	10.0 µg	6 11 5	7 6 9	19 24 19	37 34 27	92 90 92	103 106 116
	33.3 µg	4 4 12	11 11 8	17 13 35	24 33 29	120 103 95	101 104 103
	100.0 µg	8 9 8	6 11 9	19 20 17	22 27 30	88 82 86	127 119 111
	333.3 µg pptn	4 2 3	12 16 6	13 8 16	30 15 19	82 88 105	128 146 125
	1.0 mg pptn	7 7 7	5 8 6	20 18 22	23 30 34	103 92 103	90 95 125
	3.3 mg pptn	7STL 9STL *STL	12STL *STL *STL	*STL *STL *STL	*STL *STL 20STL	105STL 125STL 96STL	85STL *STL *STL

STL = slightly thin lawn
contam = contamination
pptn = precipitation
* = precipitate too dense to enable accurate counting

TABLE 27

Saccharomyces cerevisiae - Toxicity Test

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Ethyl centralite

Activation: None

Date plated: 12 December 1978

Liver preparation date: -

Date counted: 21 December 1978

Numbering colour: Black

Substance	Quantity per Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Total	% Survival
Dimethyl-sulphoxide	200 µl	131	101	132	131	113	608	100
Ethyl centralite	2.0 mg	110	74	92	100	contam	376	77
	4.0 mg	99	89	91	92	86	457	75
	8.0 mg	110	69	76	77	81	413	67
	16.0 mg	61	76	86	103	80	406	66
	32.0 mg	99	90	77	76	101	443	72
	64.0 mg	143	116	158	152	116	685	100
	83.3 mg	112	147	111	111	110	591	97

contam = contamination

TABLE 28

Saccharomyces cerevisiae - Toxicity Test

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Ethyl centralite

Activation: Aroclor-induced Fischer rat

Date plated: 12 December 1978

Liver preparation date: 11 December 1978

Date counted: 21 December 1978

Numbering colour: Blue

Substance	Quantity per Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Total	% Survival
Dimethyl-sulphoxide	200 µl	40	32	28	36	24	160	100
Ethyl centralite	2.0 mg	42	63	63	55	69	292	100
	4.0 mg	18	20	21	25	16	100	62
	8.0 mg	56	60	39	54	52	211	100
	16.0 mg	19	18	16	19	21	93	58
	32.0 mg	72	84	86	76	67	385	100
	64.0 mg	100	76	91	91	79	437	100
	83.3 mg	30	80	79	88	73	350	100

TABLE 29

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Ethyl centralite

Activation: Aroclor-induced Fischer rat

Date plated: 16 January 1979

Liver preparation date: 16 January 1979

Date counted: 22 January 1979

Batch no. (plates): R 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	180 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	114	126	99	102	102
Ethyl centralite	1.0 mg (1.8 mM)	88	109	61	61	49
	44.1 mg (82.1 mM)	122	92	116	93	96
	88.3 mg (164.5 mM)	113	131	119	99	106

Conclusion: Dose range:
Incubation time:
Re-test at lower doses

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	180 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	130	115	109	95	90
Ethyl centralite	1.0 mg (1.8 mM)	29	23	14	15	13
	44.1 mg (82.1 mM)	72	73	82	85	55
	88.3 mg (164.5 mM)	59	35	59	48	39

Conclusion: Dose range:
Incubation time:
Re-test at lower doses

TABLE 30

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: Ethyl centralite

Activation: Aroclor-induced Fischer rat

Date plated: 26 January 1979

Liver preparation date: 16 January 1979

Date counted: 1 February 1979

Batch no. (plates): M 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	151	165	159	168	153
Ethyl centralite	200 µg (0.3 mM)	110	116	174	118	106
	1 mg (1.8 mM)	68	71	65	69	59
	40 mg (74.5 mM)	85	89	86	77	74

Conclusion: Dose range: 2 mg, 1 mg, 800 µg, 600 µg, 400 µg, 200 µg, 100 µg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	181	169	145	151	110
Ethyl centralite	200 µg (0.3 mM)	91	162	111	154	111
	1 mg (1.8 mM)	46	29	31	20	20
	40 mg (74.5 mM)	49	42	64	44	57

Conclusion: Dose range: 2 mg, 1 mg, 800 µg, 600 µg, 400 µg, 200 µg, 100 µg
Incubation time: 2 h

TABLE 31

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: Ethyl centralite

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System B

Date plated: 17 July 1979

Date counted: 23 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	1500	5×10^4	7.5×10^7/ml
Ethyl centralite	$B7_{100}$ µg	1091	7.5×10^4	8.2×10^7/ml
	$B6_{200}$ µg	1374	5×10^4	6.8×10^7/ml
	$B5_{400}$ µg	1233	3.5×10^4	4.3×10^7/ml
	$B4_{600}$ µg	57	3×10^3	1.7×10^5/ml
	$B3_{800}$ µg	71	2×10^3	1.4×10^5/ml
	$B2_{1}$ mg	120	1.5×10^3	1.8×10^5/ml
	$B1_{2}$ mg pptn	1608	3.5×10^4	5.6×10^7/ml

pptn = precipitation

TABLE 31 (continued)

Conclusion:-	Doses for full test	Dilution factor
B7	100 µg	5×10^4
B6	200 µg	2×10^4
B5	400 µg	1×10^4
B4	600 µg	1×10^2
B3	800 µg	1×10^2
B2	1 mg	1×10^2
B1	2 mg	2×10^4

TABLE 32

Saccharomyces cerevisiae D5 Recombination, without activation

Project No	:	410110
Contractor	:	US Army
Operators	:	Rowan Hastwell
		Colin Riach
Substance	:	Ethyl centralite
Incubation time	:	2 h
Date plated	:	2 February 1979
Date counted	:	14 February 1979
Plates (batch)	:	M27540

Table 32 (continued)

Saccharomyces cerevisiae D5 Recombination, without activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	22981	100	0.4 (1)	0	1.3 (3)	0.4 (1)	0	0	1.3 (3)	0.4 (1)	3.4 (8)
EMS	Dose 8 control 20 mg 80.5 mM	19253	83.7	19.2 (37)	12.5 (24)	19.7 (38)	3.1 (6)	42.1 (81)	16.1 (31)	36.4 (70)	31.7 (61)	149.1 (287)
Ethyl centralite	Dose 7 100 µg 0.1 mM	19232	83.6	0.6 (2)	0.3 (1)	1.1 (4)	0.6 (2)	0.3 (1)	0	0	1.6 (3)	5.2 (10)
	Dose 6 200 µg 0.3 mM	17808	77.5	0.6 (1)	0	1.1 (2)	0	1.1 (2)	1.1 (2)	0	0.6 (1)	3.9 (7)
	Dose 5 400 µg 0.7 mM	8721	37.9	0	2.3 (2)	1.1 (1)	1.1 (1)	0	0	0	2.3 (2)	4.5 (4)
	Dose 4 600 µg 1.1 mM	2254	9.8	0	0	4.4 (1)	0	0	0	0	0	4.4 (1)
	Dose 3 800 µg 1.5 mM	3516	15.3	0	2.8 (1)	2.8 (1)	0	0	0	0	2.8 (1)	5.6 (2)
	Dose 2 1.0 mg 1.8 mM	3069	13.4	0	0	0	3.3 (1)	0	3.3 (1)	0	0	6.6 (2)
	Dose 1 2.0 mg 3.7 mM	3984	17.3	0	0	5.0 (2)	2.5 (1)	0	0	0	0	7.5 (3)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide

EMS = Ethyl methanesulphonate

TABLE 33

Saccharomyces cerevisiae D5 Recombination, with activation

Project No	:	410110
Contractor	:	US Army
Operators	:	Rowan Hastwell
		Anne Gilroy
Substance	:	Ethyl centralite
Incubation time	:	2 h
Activation	:	Aroclor-induced Fischer Rat
Liver preparation date	:	16 January 1979
Date plated	:	6 February 1979
Date counted	:	16 February 1979
Plates (batch)	:	M27540

TABLE 33 (continued)

Saccharomyces cerevisiae D5 Recombination, with activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	5520	100.0	0	1.8 (1)	0	1.8 (1)	3.6 (2)	0	7.2 (4)	1.8	14.4
DMN	Dose 8 +ve control 20 mg 267.3mM	6135	114.4	1.6 (1)	0	1.6 (1)	0	4.9 (3)	1.6 (1)	6.5 (4)	1.6	16.2
Ethyl centralite	Dose 7 100 µg 0.1 mM	6526	118.2	3.1 (2)	0	3.1 (2)	0	3.1 (2)	3.1 (2)	3.1 (2)	3.1	15.5
	Dose 6 200 µg 0.3 mM	6312	114.3	0	3.2 (2)	4.7 (3)	1.6 (1)	1.6 (1)	0	1.6 (1)	3.2	12.7
	Dose 5 400 µg 0.7 mM	5728	103.7	0	0	5.2 (3)	1.7 (1)	1.7 (1)	0	7.0 (4)	0	15.6
	Dose 4 600 µg 1.1 mM	4737	85.8	2.1 (1)	2.1 (1)	2.1 (1)	2.1 (1)	2.1 (1)	0	4.2 (2)	4.2	14.7
	Dose 3 800 µg 1.5 mM	2286	41.4	0	0	4.4 (1)	0	4.4 (1)	0	0	0	8.8
	Dose 2 1.0 mg 1.8 mM	591	10.7	0	0	0	0	16.9 (1)	0	0	0	16.9
	Dose 1 2.0 mg 3.7 mM	86	1.5	0	0	116.2 (1)	116.2 (1)	0	0	0	0	232.4

Figures in parentheses are actual number of aberrants counted
DMSO = Dimethylsulphoxide
DMN = Dimethylnitrosamine

TABLE 34

S. cerevisiae D5 Recombinogenic Activity with Ethyl centralite, with Metabolic activation Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Ethyl centralite
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	18 July 1979
Date plated:	8 August 1979
Date counted:	17 August 1979
Plates (Batch):	R16040/R43341

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	33.8 mg	1×10^5
Ethyl centralite	100 µg	5×10^4
Ethyl centralite	200 µg	2×10^4
Ethyl centralite	400 µg	1×10^4
Ethyl centralite	600 µg	1×10^2
Ethyl centralite	800 µg	1×10^2
Ethyl centralite	1 mg	1×10^2
Ethyl centralite	2 mg	2×10^4

TABLE 34 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with Ethyl centralite with metabolic activation
Modified Incubation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	$\frac{17495}{8.7 \times 10^7}$	100	0.6 (1)	1.1 (2)	1.7 (3)	1.1 (2)	1.7 (3)	0.6 (1)	0	1.7 (3)	6.9 (12)
Cyclo-phospha-mide	Dose 8 +ve control 33.8 mg	$\frac{12999}{1.3 \times 10^8}$	149	5.4 (7)	0.8 (1)	1.5 (2)	0.8 (1)	4.6 (6)	1.5 (2)	0 (0)	6.2 (8)	14.6 (19)
Ethyl centra-lite	Dose 7 100.0 µg	$\frac{14278}{7.1 \times 10^7}$	82	1.4 (2)	0	4.9 (7)	0	6.3 (9)	0	0.7 (1)	1.4 (2)	13.3 (19)
	Dose 6 200.0 µg	$\frac{24444}{4.9 \times 10^7}$	56	0.4 (1)	0	0.8 (2)	0.8 (2)	1.6 (4)	0	0.4 (1)	0.4 (1)	4.0 (10)
	Dose 5 400.0 µg	$\frac{23804}{2.4 \times 10^7}$	28	0.4 (1)	0	2.5 (6)	0.4 (1)	2.1 (5)	0.4 (1)	0.4 (1)	0.4 (1)	6.3 (15)
	Dose 4 600.0 µg	$\frac{24779}{2.5 \times 10^5}$	<1	0.4 (1)	0	1.6 (4)	0.4 (1)	0	0	0	0.4 (1)	2.4 (6)
	Dose 3 800.0 µg	*										
	Dose 2 pptn 1.0 mg	$\frac{4838}{4.8 \times 10^4}$	<1	0	0	2.0 (1)	0	0	4.1 (2)	10.3 (5)	0	16.4 (8)
	Dose 1 pptn 2.0 mg	$\frac{21372}{4.3 \times 10^7}$	49.4	0.9 (2)	0.5 (1)	0.5 (1)	1.4 (3)	0.9 (2)	0.9 (2)	0	0.9 (2)	5.1 (11)

Figures in parentheses are actual number of aberrants counted
pptn = precipitation
*Plates at this dose level contained far too many colonies to enable accurate scoring and counting

TABLE 35

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: 2-Nitrodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red with S-9
Blue without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
2-Nitrodiphenylamine	10.0 mg pptn	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-

Measurements in mm Diameter of hole = 15 mm

Controls as for Table 21

pptn = precipitation

TABLE 36

DNA Repair Test in Suspension

Project No: 410110
Contractor: U.S. Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 31 January 1979
Date examined: 1 February 1979

Substance: 2-Nitrodiphenylamine
Activation: -
Liver preparation date: -
Batch no. (plates): P30141
Numbering colour Blue

In Suspension	Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide	100 µl	997	1016	1156	1141	1103	1162
Ethyl methanesulphonate	10 µl	593	445	545	0	0	0
2-Aminofluorene	6.5 µg	1048	1013	1040	1030	1043	966
Tube I.D.	Quantity						
G	10.0 µg	574	743	884	900	721	830
F	33.3 µg	924	849	832	987	992	948
E	100.0 µg	917	786	771	1094	1084	1052
D	333.3 µg pptn	765	977	914	1024	1002	1035
C	1.0 mg pptn	893	885	891	573	574	946
B	3.3 mg pptn	752	519	799	745	726	796
A	10.0 mg pptn	803	835	947	839	918	841

pptn = precipitation

TABLE 37

DNA Repair Test in Suspension

Project No: 410110
Contractor: U.S. Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 31 January 1979
Date examined: 1 February 1979

Substance: 2-Nitrodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour Red

In Suspension		Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide		50 µl	1071	1371	1521	1147	1138	1260
Ethyl methanesulphonate		10 µl	1059	1101	1035	5	7	2
2-Aminofluorene		6.5 µg	1344	1474	1445	1256	1071	1240
Tube I.D.	Quantity							
G	10.0 µg		1354	1346	1314	1207	1046	1079
F	33.3 µg		1260	1401	1360	1243	1163	1035
E	100.0 µg		1246	1528	1637	1191	1222	1118
D	333.3 µg pptn		1394	1193	1320	1132	1354	1271
C	1.0 mg pptn		1307	1334	1155	1267	1191	1168
B	3.3 mg pptn		1358	1096	1143	1181	1192	1191
A	10.0 mg pptn		1270	1329	1354	1155	1198	1247

pptn = precipitation

TABLE 38

Toxicity Test in Strain TA 98

Substance: 2-Nitrodiphenylamine

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 25 May 1978

Operator(s): Rowan Hastwell
Batch no. (plates): R67440

Anne Gilroy
Numbering colour(s): Red = with S-9

Date plated: 4 December 1978
Blue = without S-9

Date counted: 6 December 1978
Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
2-Nitrodiphenylamine	10.0 µg	38	29
	33.3 µg	29	23
	100.0 µg	23	25
	333.3 µg	23	22
	1.0 mg pptn	6	17
	3.3 mg pptn	6	31
	10.0 mg*		

pptn = precipitation
*precipitation too dense to enable accurate counting

TABLE 39

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: 2-Nitrodiphenylamine

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 19 December 1978
Date counted: 21 December 1978

Activation: Aroclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): R67440
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	16 9 15	8 18 8	28 25 17	24 17 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	15 18 22	149 145 170	92 114 187	307 275 283
2-Nitrodiphenylamine	3.3 µg	21 11 13	12 17 14	24 26 32	20 25 21
	10.0 µg	15 18 20	17 21 17	26 34 33	21 17 20
	33.3 µg	15 14 22	10 21 23	26 22 22	19 21 24
	100.0 µg	20 16 18	15 17 9	26 32 18	15 21 26
	333.3 µg pptn	20 7 19	16 21 26	28 14 29	20 23 21
	1.0 mg pptn	4 11 6	9 19 7	21 15 30	20 40 33
	3.3 mg pptn	2STL 3STL 2STL	6STL 7STL 5STL	14 8 5	26 29 30

pptn = precipitation: STL = slightly thin lawn

TABLE 40

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: 2-Nitrodiphenylamine

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): P67542

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 9 January 1979

Blue = without S-9

Date counted: 11 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	5 11 2	7 2 2	18 17 16	16 22 19	112 122 90	102 99 86
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	25 18 20	423 394 470	120 77 104	347 382 406	223 180 203	268 255 274
2-Nitrodiphenylamine	3.3 µg	8 6 9	6 5 8	22 16 15	23 28 30	122 112 116	112 107 103
	10.0 µg	13 5 8	7 4 8	16 17 14	20 22 29	104 90 101	97 118 90
	33.3 µg	7 9 6	6 8 5	23 22 17	29 31 31	137 119 126	91 146 111
	100.0 µg	6 5 5	5 9 13	23 19 16	23 24 29	114 118 107	112 125 96
	333.3 µg pptn	7 4 5	5 12 7	9 19 18	25 37 37	123 105 125	111 115 126
	1.0 mg pptn	3 4 8	6 8 6	8 15 9	24 27 24	91 100 100	111 107 117
	3.3 mg pptn	3STL 3STL 3STL	4TL 5TL 2TL	17 8 11	23STL 17STL 23STL	103 116 94	138STL 90STL 113STL

STL = slightly thin lawn
TL = thin lawn
pptn = precipitation

TABLE 41

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: 2-Nitrodiphenylamine

Activation: Aroclor-induced Fischer rat

Date plated: 17 January 1979

Liver preparation date: 16 January 1979

Date counted: 23 January 1979

Batch no. (plates): P 53917

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	64	88	106	101	97
2-Nitro-diphenylamine	333.3 µg (0.7 mM)	86	83	100	94	112
	55.7 mg (130.0 mM)	92	106	106	105	94
	111.5 mg (260.2 mM)	113	119	110	99	110

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	105	124	113	117	97
2-Nitro-diphenylamine	333.3 µg (0.7 mM)	95	119	108	106	109
	55.7 mg (130.0 mM)	104	92	105	116	90
	111.5 mg (260.2 mM)	130	116	107	118	105

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 42

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Project no: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Jennifer Harvey
Date plated: 17 July 1979
Date counted: 23 July 1979

Substance: 2-Nitrodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): BO1441
Numbering System R

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	1500	5×10^4	7.5×10^7/ml
2-Nitro-diphenylamine	$R3_{1.35}$ mg pptn	1344	5×10^4	6.7×10^7/ml
	$R2_{13.8}$ mg pptn	776	5×10^4	3.9×10^7/ml
	$R1_{27.7}$ mg pptn	798	5×10^4	3.9×10^7/ml

pptn = precipitation

TABLE 42 (continued)

Conclusion:-	Doses for full test	Dilution factor
	R7 375 µg	5×10^4
	R6 750 µg	5×10^4
	R5 1.5 mg	5×10^4
	R4 3 mg	4×10^4
	R3 6 mg	3×10^4
	R2 12 mg	2×10^4
	R1 24 mg	2×10^4

TABLE 43

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with 2-nitrodiphenylamine

Project No:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Anne Gilroy
Substance:	2-Nitrodiphenylamine
Incubation time:	2 h
Activation:	without activation
Liver preparation date:	-
Date plated:	16 February 1979
Date counted:	28 February 1979
Plates (Batch):	R91044

TABLE 43 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity without activation, with 2-nitrodiphenylamine

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	23,386	100	0.4 (1)	0	1.3 (3)	0	0.4 (1)	0	0.9 (2)	0.4 (1)	3.0 (7)
EMS	Dose 8 +ve control 20 mg 80.5mM	21,081	90.1	28.5 (60)	4.3 (9)	19.9 (42)	5.7 (12)	42.2 (89)	29.9 (63)	65.0 (137)	32.8 (69)	195.5 (412)
2-Nitro-diphenyl-amine	Dose 7 2 mg 4.7mM	23,064 pptn	98.6	0	0	0.9 (2)	0.9 (2)	0.4 (1)	0.4 (1)	1.3 (3)	0	3.9 (9)
	Dose 6 4 mg 9.3mM	21,546 pptn	92.1	0	0	0.5 (1)	0.5 (1)	1.9 (4)	0.5 (1)	0	0	3.4 (7)
	Dose 5 8 mg 18.7mM	20,085 pptn	85.9	0.5 (1)	0	1.5 (3)	1.0 (2)	0.5 (1)	0.5 (1)	0	0.5 (1)	4.0 (8)
	Dose 4 16 mg 37.3mM	23,127 pptn	98.9	0.4 (1)	0.4 (1)	1.3 (3)	0.4 (1)	0.9 (2)	0.9 (2)	1.3 (3)	0.8 (2)	5.6 (13)
	Dose 3 32 mg 74.7mM	9,271 pptn	39.6	1.1 (1)	0	0	0	3.2 (3)	1.1 (1)	17.2 (16)	1.1 (1)	22.6 (21)
	Dose 2 64 mg 149.3mM	22,197 pptn	94.9	0	0.9 (2)	1.4 (3)	0.5 (1)	1.4 (3)	0	2.3 (5)	0.9 (2)	6.5 (14)
	Dose 1 104.9mg 244.8mM	25,363 pptn	108.5	0.8 (2)	0	1.6 (4)	0.4 (1)	0.8 (2)	0.8 (2)	0.8 (2)	0.8 (2)	5.2 (13)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphanate pptn = precipitation

TABLE 44

S. cerevisiae D5 Recombinogenic Activity with 2-Nitrodiphenylamine, with Metabolic activation Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Christopher Corden Jennifer Harvey
Substance:	2-Nitrodiphenylamine
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	15 August 1979
Date plated:	4 September 1979
Date counted:	14 September 1979
Plates (Batch):	M47142

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
2-Nitrodiphenylamine	375 µg	5×10^4
2-Nitrodiphenylamine	750 µg	5×10^4
2-Nitrodiphenylamine	1.5 mg	5×10^4
2-Nitrodiphenylamine	3 mg	4×10^4
2-Nitrodiphenylamine	6 mg	3×10^4
2-Nitrodiphenylamine	12 mg	2×10^4
2-Nitrodiphenylamine	24 mg	2×10^4

TABLE 45

Saccharomyces cerevisiae D5 Recombinogenic Activity
with 2-Nitrodiphenylamine, with metabolic activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	4408 / 2.2×10^7	100	0	0	4.5 (2)	6.8 (3)	4.5 (2)	2.3 (1)	0	0	18.1 (8)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	6146 / 6.1×10^7	277	1.6 (1)	6.5 (4)	1.6 (1)	1.6 (1)	4.9 (3)	3.3 (2)	0	8.1 (5)	19.5 (12)
2-Nitro-diphenyl-amine	Dose 7 375 µg	7120 / 3.6×10^7	164	0	0	2.8 (2)	1.4 (1)	7.0 (5)	0	0	0	11.2 (8)
	Dose 6 750 µg	6980 / 3.5×10^7	159	1.4 (4)	0	5.7 (4)	2.9 (2)	2.9 (2)	1.4 (1)	0	1.4 (1)	14.3 (10)
	Dose 5 1.5 mg pptn	6194 / 3.1×10^7	141	0	0	1.6 (1)	1.6 (1)	3.2 (2)	1.6 (1)	0	0	8.1 (5)
	Dose 4 3.0 mg pptn	6117 / 2.4×10^7	109	0	1.6 (1)	0	0	0	0	0	1.6 (1)	1.6 (1)
	Dose 3 6.0 mg pptn	7603 / 2.3×10^7	105	2.6 (2)	0	1.3 (1)	0	1.3 (1)	0	0	2.6 (2)	53 (4)
	Dose 2 12.0 mg pptn	9897 / 2.0×10^7	91	0	2.0 (2)	4.0 (4)	3.0 (3)	2.0 (2)	1.0 (1)	2.0 (2)	2.0 (2)	14.1 (14)
	Dose 1 24.0 mg pptn	10351 / 2.1×10^7	95	1.0 (1)	1.0 (1)	4.8 (5)	2.9 (3)	2.9 (3)	1.0 (1)	0	2.0 (2)	13.5 (14)

Figures in parentheses are actual number of aberrants counted
pptn = precipitation

TABLE 46

E. coli Toxicity Test

Project no: 410110
Contractor: U.S. Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Lead salicylate
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red with S-9
Blue without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Lead salicylate	10.0 mg pptn	with S-9	24 n	24 n	23 n	20 n	21 n	21 n
		without S-9	22 n	24 n	24 n	23 n	21 n	23 n

Measurements in mm Diameter of hole = 15 mm

Controls as for Table 21

pptn = precipitation

n = non specific killing

TABLE 47

Toxicity Test in Strain TA 98

Substance: Lead salicylate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 25 May 1978

Operator(s): Rowan Hastwell

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 4 December 1978

Blue = without S-9

Date counted: 6 December 1978

Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
Lead salicylate	10.0 µg	38	33
	33.3 µg	37	18
	100.0 µg	35	17
	333.3 µg	31	18
	1.0 mg	26	22
	3.3 mg pptn	0	1
	10.0 mg pptn	0	0

pptn = precipitation

TABLE 48

Salmonella Plate Test in Strains TA 1535 and TA 98

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 19 December 1978
Date counted: 21 December 1978

Substance: Lead salicylate
Activation: Aroclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): R67440
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	16 9 15	8 18 8	28 25 17	24 17 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	15 18 22	149 145 170	92 114 187	307 275 283
Lead salicylate	3.3 µg	15 12 17	13 21 15	20 21 15	17 18 20
	10.0 µg	17 13 16	14 12 16	21 15 23	17 16 21
	33.3 µg	16 12 17	12 22 14	20 19 21	21 23 22
	100.0 µg	14 17 14	13 18 19	21 30 18	13 15 14
	333.3 µg	11 11 10	8 6 8	20 15 16	19 22 16
	1.0 mg pptn	1 1 1	2 1 0	3 4 15	6 9 5
	3.3 mg pptn	0TL 0TL 0TL	VTL VTL VTL	0STL 0STL 0STL	VTL VTL VTL

pptn = precipitation: STL = slightly thin lawn:
TL = thin lawn: VTL = very thin lawn

TABLE 49

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: Lead salicylate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): P67542

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 9 January 1979

Blue = without S-9

Date counted: 11 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	5 11 2	7 2 2	18 17 16	16 22 19	112 122 90	102 99 86
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	25 18 20	423 394 470	120 77 104	347 382 406	223 180 203	268 255 274
Lead salicylate	3.3 µg	5 8 12	8 6 7	25 12 20	27 22 26	111 101 119	145 111 117
	10.0 µg	7 11 8	8 9 4	18 15 11	33 27 24	84 94 91	118 100 128
	33.3 µg	2 4 7	7 14 7	16 18 18	30 34 33	99 96 118	129 104 114
	100.0 µg	1 7 2	6 9 13	17 17 16	25 37 31	86 90 83	103 115 136
	333.3 µg	1 1 1	3 7 3	4 1 3	30 19 20	4 3 8	107 78 78
	1.0 mg pptn	0 0 0	0 2 1	0 0 0	24 20 6	0 0 0	1 0 0
	3.3 mg pptn	0 0 0	1 0 0	0 0 0	9SC 2SC 0SC	0SC 0SC 0SC	11SC 19SC 14SC

pptn = precipitation
SC = small background lawn colonies

TABLE 50

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell, Anne Gilroy

Substance: Lead salicylate

Activation: Aroclor-induced Fischer rat

Date plated: 17 January 1979

Liver preparation date: 16 January 1979

Date counted: 23 January 1979

Batch no. (plates): P 53917

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	86	not sampled	134	104	89
Lead salicylate	1.0 mg (1.0 mM)	93	not sampled	95	93	83
	53.1 mg (55.1 mM)	0	0	0	0	0
	(106.2 mg) (110.3 mM)	0	<1	0	0	0

Conclusion: Dose range: 32 mg, 24 mg, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg
Incubation time: 30 min

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	114	contam-inated	113	144	89
Lead salicylate	1.0 mg (1.0 mM)	139	contam-inated	138	169	96
	53.1 mg (55.1 mM)	0	0	0	0	0
	106.2 mg (110.3 mM)	<1	<1	0	0	<1

Conclusion: Dose range: 32 mg, 24 mg, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg
Incubation time: 30 min

TABLE 51

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: Lead salicylate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System F

Date plated: 18 July 1979

Date counted: 24 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	1455	5×10^4	7.3×10^7/ml
Lead salicylate	$F7_1$ mg	1871	5×10^4	9.4×10^7/ml
	$F6_2$ mg	2189	5×10^4	1.1×10^8/ml
	$F5_4$ mg pptn	1726	5×10^4	8.6×10^7/ml
	$F4_8$ mg pptn	1381	2×10^4	2.8×10^7/ml
	$F3_{16}$ mg pptn	14	1×10^4	1.4×10^5/ml
	$F2_{24}$ mg pptn	5	1×10^4	5×10^4/ml
	$F1_{32}$ mg pptn	0	1×10^4	-

pptn = precipitation

TABLE 51 (continued)

Conclusion:	Doses for full test	Dilution factor
	F7 500 µg	5×10^4
	F6 1 mg	5×10^4
	F5 2 mg	5×10^4
	F4 4 mg	5×10^4
	F3 8 mg	1.5×10^4
	F2 16 mg	7×10
	F1 24 mg	2×10

TABLE 52

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with lead salicylate

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach
	Anne Gilroy
Substance:	Lead salicylate
Incubation time:	30 min
Activation:	-
Liver preparation date:	-
Date plated:	20 February 1979
Date counted:	2 March 1979
Plates (Batch):	R91044

TABLE 52 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity without activation, with lead salicylate

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control [illegible] µl	19,913	100	0	1.0 (2)	4.0 (8)	0	1.0 (2)	2.0 (4)	1.0 (2)	1.0 (2)	9.0 (18)
EMS	Dose 8 +ve control 20 mg 80.5mM	19,775	99.3	18.7 (37)	30.3 (60)	3.0 (6)	1.0 (2)	51.1 (101)	19.7 (39)	64.2 (127)	49.0 (97)	188.0 (372)
Lead salicylate	Dose 7 1 mg 1.0mM	20,444	102.7	0	0	3.4 (7)	1.0 (2)	0	2.0 (4)	3.4 (7)	0	9.8 (20)
	Dose 6 2 mg 2.1mM	18,652 pptn	93.7	0	0	2.1 (4)	0	2.1 (4)	0	2.7 (5)	0	6.9 (13)
	Dose 5 4 mg 4.2mM	18,362 pptn	92.2	0	2.2 (4)	1.6 (3)	0	1.6 (3)	1.1 (2)	3.8 (7)	2.2 (4)	10.3 (19)
	Dose 4 8 mg 8.3mM	32,233 pptn	161.9	1.2 (4)	0.9 (3)	2.8 (9)	0.9 (3)	0.3 (1)	0	0.9 (3)	2.1 (7)	7.0 (23)
	Dose 3 16 mg 16.6mM	13,889 pptn	69.7	0	0.7 (1)	4.3 (6)	0	3.6 (5)	0.7 (1)	2.1 (3)	0.7 (1)	11.4 (16)
	Dose 2 24 mg 24.9mM	10,552 pptn	53.0	0.9 (1)	1.9 (2)	4.7 (5)	0	7.6 (8)	2.8 (3)	0.9 (1)	2.8 (3)	18.8 (20)
	Dose 1 32 mg 33.2mM	6,333 pptn	31.8	0	3.1 (2)	9.5 (6)	0	3.1 (2)	1.6 (1)	7.9 (5)	3.1 (2)	25.2 (16)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 53

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with lead salicylate (Re-test)

Project No:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Anne Gilroy
Substance:	Lead salicylate
Incubation time:	30 min
Activation:	-
Liver preparation date:	-
Date plated:	9 March 1979
Date counted:	21 March 1979
Plates (Batch):	R40341

TABLE 53 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity without activation, with lead salicylate (Re-test)

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 100 µl	25,518	100	0.8 (2)	1.2 (3)	1.6 (4)	0.8 (2)	2.4 (6)	1.2 (3)	2.4 (6)	2.0 (5)	10.4 (26)
EMS	Dose 8 +ve control 20 mg 80.5mM	25,092	98.3	27.5 (69)	17.9 (45)	14.7 (37)	4.0 (10)	53.8 (135)	43.0 (108)	73.3 (184)	45.4 (114)	185.8 (588)
Lead salicylate	Dose 7 20 mg 20.8mM	20,289 pptn	79.5	0.5 (1)	1.5 (3)	1.0 (2)	0.5 (1)	1.5 (3)	1.5 (3)	3.5 (7)	2.0 (4)	10.0 (20)
	Dose 6 30 mg 31.2mM	611 pptn	2.4	0	0	0	0	32.7 (2)	16.4 (1)	16.4 (1)	0	65.5 (4)
	Dose 5 40 mg 41.6mM	2 pptn	<0.1	0	0	0	0	0	0	0	0	0
	Dose 4 50 mg 51.9mM	0 pptn	0	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 54

S. cerevisiae D5 Recombinogenic Activity
with Lead Salicylate with Metabolic Activation,
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Anne Scott
	Colin Riach
Substance:	Lead Salicylate
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer Rat
Liver preparation date:	18 July 1979
Date plated:	14 August 1979
Date counted:	24 August 1979
Plates (Batch)	R43341

Dilution Factors used to dilute incubation tubes
after 18 h incubation

Substance	Dose		Dilution Factor
Dimethylsulphoxide	100	µl	5×10^4
Cyclophosphamide	40	mg	1×10^5
Lead Salicylate	500	µg	5×10^4
Lead Salicylate	1	mg	5×10^4
Lead Salicylate	2	mg	5×10^4
Lead Salicylate	4	mg	5×10^4
Lead Salicylate	8	mg	1.5×10^4
Lead Salicylate	16	mg	7×10
Lead Salicylate	24	mg	2×10

TABLE 54 (continued)

S. cerevisiae D5 Recombinogenic Activity
with Lead Salicylate, with Metabolic Activation,
Modified Incubation

Substance	Dose	Colonies counted: Viable count per ml after 18 h incubation	Survival	No. of aberrants/10^4 survivors: Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline	Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	6,669 / 3.3×10^7	100	0	0	1.5 (1)	0	6.0 (4)	0	3.0 (2)	0	10.5 (7)
Cyclo-phos-phamide	Dose 8 +ve control 40.0 mg 72 mM	5,859 / 5.8×10^7	176	5.1 (3)	6.8 (4)	1.7 (1)	1.7 (1)	6.8 (4)	3.4 (2)	3.4 (2)	11.9 (7)	29.0 (17)
Lead Salicy-late	Dose 7 500 µg 0.5 mM	8,168 / 4.1×10^7	124	0	1.2 (1)	2.4 (2)	1.2 (1)	2.4 (2)	0	0	1.2 (1)	7.3 (6)
	Dose 6 1 mg 1 mM	6,844 / 3.4×10^7	103	0	0	4.4 (3)	0	2.9 (2)	1.5 (1)	1.5 (1)	0	10.2 (7)
	Dose 5 2 mg 2 mM	6,811 / 3.4×10^7	103	1.5 (1)	0	0	4.4 (3)	7.3 (5)	0	1.5 (1)	1.5 (1)	14.7 (10)
	Dose 4 4 mg 4 mM	43 / 2.2×10^5	1	0	0	0	0	0	0	0	0	0
	Dose 3 8 mg 8 mM	0 / 0	0 pptn	0	0	0	0	0	0	0	0	0
	Dose 2 16 mg 17 mM	1 / 7.0	1 pptn	0	0	0	0	0	0	0	0	0
	Dose 1 24 mg 25 mM	35 / 7.0×10	1 pptn	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted

TABLE 55

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Lead resorcylate
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Lead resorcylate	10.0 mg pptn	with S-9	39 n	40 n	40 n	35 n	36 n	36 n
		without S-9	40 n	39 n	40 n	36 n	37 n	35 n

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
pptn = precipitation
n = non specific killing

TABLE 56

Toxicity Test in Strain TA 98

Substance: Lead resorcylate

Project no: 410110
Activation: Aroclor-induced Fischer rat
Contractor: US Army
Liver preparation date: 25 May 1978
Operator(s): Rowan Hastwell
Batch no. (plates): R67440
Anne Gilroy
Numbering colour(s): Red = with S-9
Date plated: 4 December 1978
Blue = without S-9
Date counted: 6 December 1978
Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
Lead resorcylate	10.0 µg	34	18
	33.3 µg	39	21
	100.0 µg	35	35
	333.3 µg	35	22
	1.0 mg	21	19
	3.3 mg pptn	11	15
	10.0 mg pptn	3	3

pptn = precipitation

TABLE 57

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Lead resorcylate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 19 December 1978

Blue - without S-9

Date counted: 21 December 1978

Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	16 9 15	8 18 8	28 25 17	24 17 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	15 18 22	149 145 170	92 114 187	307 275 283
Lead resorcylate	10.0 µg	16 14 17	18 18 24	18 17 25	20 22 16
	33.3 µg	18 19 16	24 16 17	17 30 22	15 14 26
	100.0 µg	22 28 26	20 14 17	21 34 25	25 19 22
	333.3 µg	19 12 22	14 20 14	25 20 23	15 21 19
	1.0 mg	8 10 18	10 11 16	21 23 24	12 18 17
	3.3 mg pptn	7 3 3	4 5 7	6 8 10	19 15 16
	10.0 mg pptn	0 0 0	0STL 0STL 0STL	0 0 0	0 0 0

pptn = precipitation: STL = slightly thin lawn

TABLE 58

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: Lead resorcylate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 16 January 1979

Operator(s): Colin Riach

Batch no. (plates): Red - M85640 Blue - P74848

Numbering colour(s): Red = with S-9

Blue = without S-9

Date plated: 17 January 1979

Date counted: 19 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	8 7 6	5 7 10	22 17 19	34 37 28	124 95 127	127 117 95
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	17 23 20	167 179 171	152 135 126	391 429 304	208 190 195	342 317 355
Lead resorcylate	10.0 µg	9 12 13	9 7 12	22 20 26	19 20 15	117 111 141	114 134 108
	33.3 µg	7 14 9	11 6 12	30 18 34	24 36 38	127 103 133	135 115 111
	100.0 µg	11 7 13	113 14 15	23 20 20	30 38 29	102 108 120	125 128 111
	333.3 µg	11 11 7	7 4 14	22 28 24	41 26 25	79 108 86	127 137 102
	1.0 mg	9 5 9	12 7 12	28 25 39	27 28 25	73 78 72	108 85 101
	3.3 mg pptn	2 2 2	5 12 5	4 3 8	23 26 22	9 6 8	36 38 37
	10.0 mg pptn	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

pptn = precipitation

TABLE 59

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Lead resorcylate

Activation: Aroclor-induced Fischer rat

Date plated: 18 January 1979

Liver preparation date: 16 January 1979

Date counted: 24 January 1979

Batch no. (plates): P 53917

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	148	116	139	118	114
Lead resorcylate	2.0 mg	144	111	contam-inated	116	117
	40.4 mg	132	108	contam-inated	109	88
	80.9 mg	1	0	<1	0	0

Conclusion: Dose range: 72 mg, 64 mg, 32, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	118	127	110	116	99
Lead resorcylate	2.0 mg	136	151	111	98	109
	40.4 mg	111	124	101	95	93
	80.9 mg	0	0	0	0	0

Conclusion: Dose range: 72 mg, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 60

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: Lead resorcylate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System H

Date plated: 18 July 1979

Date counted: 24 July 1979

Substance	Dose	Counts from 10 plates	Dilution Factor used	Viable count after 18h incubation
Dimethylsulphoxide	100 µl	1455	5×10^4	7.3×10^7/ml
Lead resorcylate	$H7_1$ mg	2063	5×10^4	1.0×10^8/ml
	$H6_2$ mg	1738	5×10^4	8.7×10^7/ml
	$H5_4$ mg pptn	1606	5×10^4	8.0×10^7/ml
	$H5_8$ mg pptn	4006	2×10^4	8.0×10^7/ml
	$H3_{16}$ mg pptn	95	1×10^4	9.5×10^5/ml
	$H2_{24}$ mg pptn	634	1×10^4	6.3×10^5/ml
	$H1_{36}$ mg pptn	472	1×10^4	4.7×10^5/ml

pptn = precipitation

TABLE 60 (continued)

Conclusion:	Doses for full test	Dilution factor
	H7 1 mg	5×10^4
	H6 2 mg	5×10^4
	H5 4 mg	5×10^4
	H4 8 mg	5×10^4
	H3 16 mg	4×10^2
	H2 24 mg	3×10^2
	H1 36 mg	2×10^2

TABLE 61

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation with lead resorcylate

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach
	Rowan Hastwell
Substance:	Lead resorcylate
Incubation time:	2 h
Activation:	-
Liver preparation date:	-
Date plated:	23 February 1979
Date counted:	9 March 1979
Plates (Batch):	M50144

TABLE 62

Saccharomyces cerevisiae D5 Recombinogenic activity without activation, with lead resorcylate

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	26008	100	0	0.4 (1)	0.4 (1)	0	0.4 (1)	0	2.3 (6)	0.4 (1)	3.5 (9)
EMS	Dose 8 +ve control 20 mg 80.5mM	19514	75.0	21.0 (41)	20.0 (39)	15.4 (30)	2.6 (5)	131.7 (257)	29.7 (58)	48.1 (94)	41.0 (80)	268.5 (524)
Lead resorcylate	Dose 7 2 mg	19689	75.7	0.5 (1)	0.5 (1)	0.5 (1)	0.5 (1)	0	0	1.0 (2)	1.0 (2)	3.0 (6)
	Dose 6 4 mg	19347 pptn	74.4	0	0	0.5 (1)	0	0	0	2.1 (4)	0	2.6 (5)
	Dose 5 8 mg	18922 pptn	72.8	0.5 (1)	0	1.1 (2)	0.5 (1)	0.5 (1)	0	0.5 (1)	0.5 (1)	3.1 (6)
	Dose 4 16 mg	19362 pptn	74.4	0	0	0	0	3.1 (6)	0.5 (1)	1.0 (2)	0	4.6 (9)
	Dose 3 32 mg	24879 pptn	95.7	0	0	1.2 (3)	0.4 (1)	0.8 (2)	0	1.6 (4)	0	4.0 (10)
	Dose 2 64 mg	28 pptn	0.1	0	0	0	0	0	0	0	0	0
	Dose 1 72 mg	35 pptn	0.1	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 63

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with lead resorcylate (Re-test)

Project No:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Anne Gilroy
Substance:	Lead resorcylate
Incubation time:	2 h
Activation:	-
Liver preparation date:	-
Date plated:	9 March 1979
Date counted:	21 March 1979
Plates (Batch):	R40341

TABLE 63 (continued)

Saccharomyces cerevisiae D5 *Recombinogenic activity without activation,*
with lead resorcylate (Re-test)

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	25518	100	0.8 (2)	1.2 (3)	1.6 (4)	0.8 (2)	2.4 (6)	1.2 (3)	2.4 (6)	2.0 (5)	10.4 (26)
EMS	Dose 8 +ve control 20 mg 80.5mM	25092	98.3	27.5 (69)	17.9 (45)	14.7 (37)	4.0 (10)	5.4 (135)	43.0 (108)	73.3 (184)	45.4 (114)	185.8 (588)
Lead resorcylate	Dose 7 30 mg	23914 pptn	93.7	0.4 (1)	0.4 (1)	1.7 (4)	0.4 (1)	2.1 (5)	0.8 (2)	1.7 (4)	0.8 (2)	7.5 (18)
	Dose 6 40 mg	9638 pptn	37.8	0	0	4.2 (4)	2.1 (2)	0	3.1 (3)	5.2 (5)	0	14.6 (14)
	Dose 5 50 mg	20 pptn	7.8	0	0	0	0	0	0	0	0	0
	Dose 4 60 mg	36 pptn	14.1	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 64

S. cerevisiae D5 Recombinogenic Activity with
Lead resorcylate, with Metabolic activation
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Christopher Corden
Substance:	Lead resorcylate
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	15 August 1979
Date plated:	24 August 1979
Date counted:	3 September 1979
Plates (Batch):	M26040 R16040

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
Lead resorcylate	1 mg	5×10^4
Lead resorcylate	2 mg	5×10^4
Lead resorcylate	4 mg	5×10^4
Lead resorcylate	8 mg	5×10^4
Lead resorcylate	16 mg	4×10^2
Lead resorcylate	24 mg	3×10^2
Lead resorcylate	36 mg	2×10^2

TABLE 64 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with Lead resorcylate, with metabolic activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	8813 / 4.4×10^7	100	0	0	1.1 (1)	1.1 (1)	2.3 (2)	1.1 (1)	0	0	5.7 (5)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	6781 / 6.8×10^7	154	2.9 (2)	0	4.4 (3)	0	2.9 (2)	0	0	2.9 (2)	10.3 (7)
Lead resor-cylate	Dose 7 1.0 mg	10333 / 5.2×10^7	118	1.0 (1)	1.0 (1)	1.0 (1)	2.9 (3)	1.0 (1)	0	0	1.9 (2)	6.8 (7)
	Dose 6 2.0 mg	10128 / 5.1×10^7	116	1.0 (1)	0	1.0 (1)	1.0 (1)	0	0	1.0 (1)	1.0 (1)	3.9 (4)
	Dose 5 4.0 mg	9953 / 5.0×10^7	114	0	1.0 (1)	2.0 (2)	0	3.0 (3)	0	0	1.0 (1)	6.0 (6)
	Dose 4 8.0 mg pptn	4326 / 2.2×10^7	50	0	2.3 (1)	0	0	4.6 (2)	0	2.3 (1)	2.3 (1)	9.2 (4)
	Dose 3 16.0 mg pptn	26428 / 1.1×10^6	2.5	0	0	0.4 (1)	0.7 (2)	1.1 (3)	1.5 (4)	0	0	4.0 (10)
	Dose 2 24.0 mg pptn	37146 / 1.1×10^7	25	0.3 (1)	0	0.3 (1)	0	0.8 (3)	0	0	0.3 (1)	1.3 (5)
	Dose 1 360 mg pptn	42424 / 8.5×10^6	19	0	0	0.7 (3)	0.2 (1)	0	0	0.2 (1)	0	1.2 (5)

Figures in parentheses are actual number of aberrants counted
pptn = precipitation

TABLE 65

E. coli Toxicity Test

Project no: 410110
Contracted: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Diethyleneglycoldinitrate
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	Rol... (100 µ...)					
Diethylene-glycoldinitrate	10.0 mg	with S-9	16 n	16 n	17 n	16 n	16 n	-
		without S-9	16 n	16 n	16 n	16 n	17 n	16 n

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
n = non specific killing

TABLE 66

Toxicity Test in Strain TA 98

Substance: Diethylglycoldinitrate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 25 May 1978

Operator(s): Rowan Hastwell

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 4 December 1978

Blue = without S-9

Date counted: 6 December 1978

Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
Diethylglycoldinitrate	10.0 µg	32	22
	33.3 µg	34	26
	100.0 µg	26	23
	333.3 µg	36	25
	1.0 mg	33	26
	3.3 mg	32	
	10.0 mg pptn	24	26

pptn = precipitation

TABLE 67

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Diethyleneglycoldinitrate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 19 December 1978

Blue = without S-9

Date counted: 21 December 1978

Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	16 9 15	8 18 8	28 25 17	24 17 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	15 18 22	149 145 170	92 114 187	307 275 283
Diethylene-glycoldinitrate	10.0 µg	7 3 4	1 0 1	8 9 9	7 12 11
	33.3 µg	4 5 8	8 11 9	15 18 19	19 23 14
	100.0 µg	2 2 3	0 1 0	3 3 2	10 12 20
	333.3 µg	4 11 5	5 8 10	32 38 32	18 14 10
	1.0 mg	11 14 6	15 12 13	23 19 36	15 24 6
	3.3 mg	4 11 13	10 14 10	contam 16 20	23 25 5
	10.0 mg pptn	13 8 9	3 13 14	15 14 2	0 0 0

pptn = precipitation: contam = contamination

TABLE 68

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: Diethyleneglycoldinitrate

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 16 January 1979

Operator(s): Colin Riach

Batch no. (plates): Red - M85640 Blue - P74848

Numbering colour(s): Red = with S-9

Blue = without S-9

Date plated: 17 January 1979

Date counted: 19 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	8 7 6	5 7 10	22 17 19	34 37 28	124 95 127	127 117 95
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	17 23 20	167 179 171	152 135 126	391 429 304	208 190 195	342 317 355
Diethylene-glycoldinitrate	10.0 µg	9 8 14	12 11 9	35 9 23	35 27 49	104 123 113	126 106 105
	33.3 µg	7 12 7	11 5 11	26 28 27	30 31 24	120 131 135	140 123 145
	100.0 µg	15 8 3	8 11 7	22 14 30	37 30 38	120 102 129	153 112 127
	333.3 µg	6 12 2	12 8 16	26 24 20	32 31 24	128 118 129	125 146 105
	1.0 mg	12 11 11	15 4 12	31 12 31	30 31 36	130 125 103	126 128 135
	3.3 mg	3 4 6	6 4 8	19 18 24	40 37 31	124 116 126	102 153 130
	10.0 mg pptn	3 3 5	5 4 8	22 17 19	14 26 51	137 127 128	74 72 67

pptn = precipitation

TABLE 69

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Diethyleneglycoldinitrate

Activation: Aroclor-induced Fischer rat

Date plated: 18 January 1979

Liver preparation date: 16 January 1979

Date counted: 24 January 1979

Batch no. (plates): P 53917

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	123	66	153	121	110
Diethylene-glycoldinitrate	10.0 mg (25.4 mM)	127	90	153	113	111
	135.5 mg (345.4 mM)	148	144	72	123	85
	271.0 mg (690.9 mM)	143	105	78	83	131

Conclusion: Dose range: Saturation, 128 mg, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	142	141	135	131	116
Diethylene-glycoldinitrate	10.0 mg (25.4 mM)	124	104	140	105	124
	135.5 mg (345.4 mM)	121	80	128	111	117
	271.0 mg (690.9 mM)	157	98	135	132	139

Conclusion: Dose range: Saturation, 128 mg, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg
Incubation time: 2 h

TABLE 70

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Project no: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Jennifer Harvey
Date plated: 18 July 1979
Date counted: 24 July 1979

Substance: Diethyleneglycoldinitrate
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): BO1441
Numbering System J

Substance	Dose	Counts from 10 plates	Dilution actor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	1455	5×10^4	7.3×10^7/ml
Diethylene-glycoldinitrate	$J3_{6.6}$ mg	1936	1×10^4	1.9×10^7/ml
	$J2_{66.7}$ mg	1746	1×10^4	1.7×10^7/ml
	$J1_{133.5}$mg	1733	1×10^4	1.7×10^7/ml

TABLE 70 (continued)

Conclusion:-	Dose for full test	Dilution factor
	J7 2 mg	1×10^4
	J6 4 mg	1×10^4
	J5 8 mg	1×10^4
	J4 16 mg	1×10^4
	J3 32 mg	9×10^3
	J2 64 mg	8×10^3
	J1 Saturation	8×10^3

TABLE 71

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Diethyleneglycoldinitrate

Project No:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Anne Gilroy
	Colin Riach
Substance:	Diethyleneglycoldinitrate
Incubation time:	2 h
Activation:	-
Liver preparation date:	-
Date plated:	16 March 1979
Date counted:	28 March 1979
Plates (Batch):	M70218

TABLE 71 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity without activation, with Diethyleneglycoldinitrate

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	7856	100	1.3 (1)	0	1.3 (1)	1.3 (1)	2.5 (2)	5.1 (4)	5.1 (4)	1.3 (1)	16.6 (13)
EMS	Dose 8 +ve control 20 mg 80.5mM	8301	105.7	15.7 (13)	20.5 (17)	9.6 (8)	4.8 (4)	112.0 (93)	98.8 (82)	196.4 (163)	36.2 (30)	457.8 (280)
Di-ethylene glycold-initrate	Dose 7 4 mg 10.2 mM	7949	101.2	0	0	3.8 (3)	2.5 (2)	2.5 (2)	1.3 (1)	11.3 (9)	0	21.4 (17)
	Dose 6 8 mg 20.4mM	6998	89.1	1.4 (1)	1.4 (1)	0	1.4 (1)	0	2.9 (2)	12.9 (9)	2.8 (2)	17.6 (14)
	Dose 5 16 mg 40.8mM	6844 pptn	87.1	1.5 (1)	0	2.9 (2)	1.5 (1)	0	2.9 (2)	8.8 (6)	1.5 (1)	17.6 (12)
	Dose 4 32 mg 81.6mM	6506 pptn	82.8	1.5 (1)	0	0	1.5 (1)	3.1 (2)	1.5 (1)	9.2 (6)	1.5 (1)	16.8 (11)
	Dose 3 64 mg 163.2mM	6599 pptn	84.1	1.5 (1)	1.5 (1)	3.0 (2)	0	1.5 (1)	4.5 (3)	6.1 (4)	3.0 (2)	18.1 (12)
	Dose 2 128 mg 326.4mM	7204 pptn	91.7	2.8 (2)	0	1.4 (1)	0	2.8 (2)	8.3 (6)	8.3 (6)	2.8 (2)	23.6 (17)
	Dose 1 288 mg 734.3mM	7529 pptn	95.8	0	2.7 (2)	1.3 (1)	0	5.3 (4)	4.0 (3)	4.0 (3)	2.7 (2)	17.3 (13)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 72

S. cerevisiae D5 Recombinogenic Activity
with Diethyleneglycoldinitrate, with Metabolic Activation,
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Jennifer Harvey Colin Riach
Substance:	Diethyleneglycoldinitrate
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer Rat
Liver preparation date:	18 July 1979
Date plated:	3 August 1979
Date counted	13 August 1979
Plates (Batch)	BO1441, P16040, R43341

Dilution Factors used to dilute incubation tubes
after 18 h incubation

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	2×10^4
Cyclophosphamide	41.33mg	3×10^4
DEGN	2 mg	2.6×10^4
DEGN	4 mg	2×10^4
DEGN	8 mg	6.7×10^3
DEGN	16 mg	2.5×10^3
DEGN	32 mg	3.3×10^3
DEGN	64 mg	3.3×10^3
DEGN	141 mg	1×10^4

TABLE 72 (continued)

S. cerevisiae D5 Recombinogenic Activity
with Diethyleneglycoldinitrate, with Metabolic Activation,
Modified Incubation

Substance	Dose	Colonies counted: Viable count per ml after 18 h incubation	% survival	No. of aberrants/10^4 survivors: Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline	Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	29,179 / 5.8×10^7	100	0.3 (1)	0	0	0.7 (2)	0	1.0 (3)	0.7 (2)	0.3 (1)	4.1 (8)
Cyclo-phos-phamide	Dose 8 +ve control 41.33mg 74 mM	26,234 / 7.9×10^7	136	0	0.4 (1)	0	0.8 (2)	1.5 (4)	0.8 (2)	1.1 (3)	0.4 (1)	4.6 (12)
Di-ethylene-glycoldi-nitrate	Dose 7 2 mg 5 mM	29,312 / 7.6×10^7	172	0	0	0.3 (1)	0.7 (2)	1.0 (3)	0	0.7 (2)	0	2.7 (8)
	Dose 6 4 mg 10 mM	25,929 / 5.2×10^7	90	0	0.4 (1)	0	0.4 (1)	1.2 (3)	1.2 (3)	0.4 (1)	0.4 (1)	3.5 (9)
	Dose 5 8 mg 20 mM	29,317 / 2.0×10^7	34	0.7 (2)	0	0.7 (2)	0.7 (2)	1.0 (3)	0	0.7 (2)	0.7 (2)	3.8 (11)
	Dose 4 16 mg 41 mM	23,920 / 6.0×10^6	10	0	0	0.4 (1)	1.3 (3)	0.8 (2)	1.7 (4)	0.4 (1)	0	4.6 (11)
	Dose 3 32 mg 82 mM	23,664 / 7.8×10^6	13 pptn	0.4 (1)	0	2.1 (5)	0	1.3 (3)	0.4 (1)	2.1 (5)	0.4 (1)	6.3 (15)
	Dose 2 64 mg 163 mM	24,358 / 8.0×10^6	14 pptn	0.4 (1)	0	1.2 (3)	0.8 (2)	0.8 (2)	0.4 (1)	0.4 (1)	0.4 (1)	4.1 (10)
	Dose 1 141.15mg 36 mM	10,813 / 1.1×10^7	19 pptn	0	0	0	0	2.7 (3)	1.8 (2)	0.9 (1)	0	5.5 (6)

Figures in parentheses are actual number of aberrants counted

TABLE 73

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Tetryl
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A⁺ (100 µl)			pol A⁻ (200 µl)		
Tetryl	10.0 mg pptn	with S-9	20 s	20 s	20 s	20 s	19 s	20 s
		without S-9	20 s	21 s	21 s	20 s	20 s	20 s

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
pptn = precipitation
s = specific killing

TABLE 74

Toxicity Test in Strain TA 98

Substance: Tetryl

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 25 May 1978

Operator(s): Rowan Hastwell

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 4 December 1978

Blue = without S-9

Date counted: 6 December 1978

Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
Tetryl	10.0 µg	39	56
	33.3 µg	39	126
	100.0 µg	138	350
	333.3 µg	541	NL
	1.0 mg pptn	NL	NL
	3.3 mg pptn	NL	NL
	10.0 mg pptn	NL	NL

pptn = precipitation
NL = no lawn i.e. complete killing

TABLE 75

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Tetryl

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R67440

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 19 December 1978

Blue = without S-9

Date counted: 21 December 1978

Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	16 9 15	8 18 8	28 25 17	24 17 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	15 18 22	149 145 170	92 114 187	307 275 283
Tetryl	1.0 µg	3 4 2	5 1 2	15 11 7	16 26 31
	3.3 µg	3 5 7	7 10 9	16 22 17	37 40 22
	10.0 µg	4 4 3	12 11 10	16 19 19	89 64 81
	33.3 µg	7 6 5	22 14 16	27 26 23	120 132 contam
	100.0 µg	4 2 0	1TL 1TL 0TL	47 51 44	76 156TL 95TL
	333.3 µg	VTL VTL VTL	NL NL NL	362 208 contam	NL NL NL
	1.0 mg pptn	NL NL NL	NL NL NL	NL NL NL	NL NL NL

pptn = precipitation: contam = contamination:
TL = thin lawn: VTL = very thin lawn: NL = no lawn i.e. complete killing

TABLE 76

Re-test of Salmonella Plate Test in Strain TA 98

Substance: Tetryl

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 11 December 1978

Operator(s): Colin Riach
Batch no. (plates): P88340

Anne Gilroy
Numbering colour(s): Red = with S-9

Date plated: 8 January 1979
Blue = without S-9

Date counted: 10 January 1979
Culture batch: C

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	22 27 21	21 18 21
2-Aminoanthracene 2-Nitrofluorene	0.5 µg 2.0 µg	110 180 137	326 318 424
Tetryl	6.25 µg	17 38 36	28 55 41
	12.5 µg	36 27 30	61 58 59
	25.0 µg	50 46 46	88 91 86
	50.0 µg	57 61 73	239 226 260
	100.0 µg	194 207 189	213TL 227TL 184TL
	200.0 µg	VTL VTL VTL	NL NL NL
	300.0 µg	NL NL NL	NL NL NL
	400.0 µg	NL NL NL	NL NL NL

TL = thin lawn
VTL = very thin lawn
NL = no lawn

TABLE 77

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: Tetryl

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: U.S. Army

Liver preparation date: 20 September 1979

Operator(s): Colin Riach

Batch no. (plates): P9040

Jennifer Harvey

Numbering colour(s): Red with S-9

Blue without S-9

Date plated: 25 September 1979

Date counted: 27 September 1979

Culture batch: D

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	17 19 16	4 13 7	15 14 28	9 9 16	74 86 85	81 77 76
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	29 30 14	1422 1122 1503	152 175 145	296 341 314	231 289 296	241 210 234
Tetryl	1.0 µg	13 20 14	49 66 83	29 39 26	30 44 25	90 91 90	117 149 108
	3.3 µg	16 14 18	137 129 151	16 30 18	49 57 62	87 76 108	267 264 216
	10.0 µg	24 40 38	216 222 221	54 51 53	91 183 150	97 100 122	1061 1052 1063
	33.3 µg	234 240 185	162 TL 164 TL 174 TL	152 103 158	234 249 243	217 199 180	518 519 554
	100.0 µg	123 111 126	VTL VTL VTL	176 159 168	TL TL TL	637 536 627	TL TL TL
	333.3 mg	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL
	1.0 mg	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL

TL = thin lawn

VTL = very thin lawn

TABLE 78

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: Tetryl

Activation: Aroclor-induced Fischer rat

Date plated: 19 January 1979

Liver preparation date: 16 January 1979

Date counted: 29 January 1979

Batch no. (plates): P 53917

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	83	92	78	85	71
Tetryl	500 µg (0.8 mM)	81	136	111	114	108
	39.7 mg (69.1 mM)	52	46	18	8	7
	79.5 mg (138.4 mM)	90	89	37	13	9

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	73	80	110	98	not sampled
Tetryl	500 µg (0.8 mM)	94	not sampled	not sampled	not sampled	101
	39.7 mg (69.1 mM)	99	33	14	9	5
	79.5 mg (138.4 mM)	74	35	21	9	6

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 79

Saccharomyces cerevisiae D5 - Toxicity Test
Without Activation with Tetryl

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Jennifer Harvey

Date plated: 19 March 1979

Date counted: 23 March 1979

Substance: Tetryl

Activation: Without activation

Liver preparation date: -

Numbering colour Black/M82041

Incubation: 2 h

Substance	Quantity per Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Total	% Survival
DMSO K1	200 µl	199,208, 190,226, 201	181,196, 167,224, 189	196,202 212,273 219	194,203, 201,208 182	230,273 239,188 281	5282	100
Tetryl K2	250 µg 0.4 mM	2,2,9, 3,3,	4,5,4, 3,5	2,3,12, 2,3	7,3,3, 3,9,	3,3,3, 4,10,	110	2.1
Tetryl K3	500 µg 0.9 mM	7,8,7, 3,5	7,5,6, 7,12,	4,5,7, 2,2,	3,6,4, 4,4,	9,8,4, 7,4,	140	2.7
Tetryl K4	750 µg 1.3 mM	2,1,2, 1,2	2,2,3, 0,0	2,1,1, 2,0,	5,2,1, 0,3,	2,3,1, 3,0,	41	0.8
Tetryl K5	1 mg 1.7 mM	2,2,1, 2,2,	4,5,4, 4,3	4,1,0 4,1,	4,6,2 0,5	2,2,0, 2,4,	68 pptn	1.3
Tetryl K6	2 mg 3.5 mM	0,0,0, 0,1,	0,1,0 2,2	0,0,1, 2,0	3,0,0, 0,1,	3,2,3, 1,2	24 pptn	0.5
Tetryl K7	16 mg 27.9 mM	6,7,7, 5,10,	10,8,10 6,12,	9,8,2, 9,7,	9,8,6, 4,16,	7,5,4, 7,7	189 pptn	3.6
Tetryl K8	64 mg 111.4 mM	7,21,11, 18,10,	7,6,17 31,17,	21,9,16 18,17,	17,21,14 15,18,	14,10,9 9,14,	367 pptn	6.9

Conclusion: Dose - 2 mg, 1 mg, 500 µg, 250 µg, 125 µg, 62.5 µg, 31.25 µg

Time - 2 h

DMSO - Dimethylsulphoxide

pptn - precipitation

TABLE 80

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: Tetryl

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System: L

Date plated: 19 July 1979

Date counted: 25 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2657	2×10^4	5.3×10^7/ml
Tetryl	L^7 31.25 µg	2791	2×10^4	5.5×10^7/ml
	L^6 62.5 µg	2836	2×10^4	5.7×10^7/ml
	L^5 125 µg	1591	1×10^4	1.6×10^7/ml
	L^4 250 µg	15	2×10^3	3×10^4/ml
	L^3 500 µg	33	2×10^3	6.6×10^4/ml
	L^2 1 mg pptn	57	1×10^3	5.7×10^4/ml
	L^1 2 mg pptn	1	1×10^4	1×10^4/ml

pptn = precipitation

TABLE 80 (continued)

Conclusion:	Doses for full test	Dilution factor
	L7 31.25 µg	5×10^4
	L6 62.5 µg	5×10^4
	L5 125 µg	1.5×10^4
	L4 250 µg	3×10
	L3 500 µg	4×10
	L2 1 mg	4×10
	L1 2 mg	1×10

TABLE 81

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Tetryl

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach
	Anne Gilroy
Substance:	Tetryl
Incubation time:	2 h
Activation:	-
Liver preparation date:	-
Date plated:	2 March 1979
Date counted:	14 March 1979
Plates (Batch):	B11004

TABLE 81 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity
without activation with Tetryl

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	32198	100	0	0	0.9 (3)	0.3 (1)	2.2 (7)	0	6.2 (20)	0	9.6 (31)
EMS	Dose 8 +ve control 20 mg 80.5mM	31752	98.6	17.3 (55)	6.2 (20)	30.9 (98)	9.1 (29)	17.9 (57)	12.6 (40)	111.2 (353)	23.5 (75)	205.2 (652)
Tetryl	Dose 7 2 mg 3.5mM	50 pptn	0.2	0	0	0	0	0	0	0	0	0
	Dose 6 4 mg 7.0mM	28 pptn	0.1	0	0	0	0	0	0	0	0	0
	Dose 5 8 mg 13.9mM	67 pptn	0.2	0	0	0	0	0	0	0	0	0
	Dose 4 16 mg 27.0mM	195 pptn	0.6	0	0	0	0	0	0	0	0	0
	Dose 3 32 mg 55.7mM	190 pptn	0.6	0	0	0	0	0	0	0	0	0
	Dose 2 64 mg 114mM	410 pptn	1.2	0	0	0	0	0	0	0	0	0
	Dose 1 128 mg 222.8mM	141 pptn	0.4	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 82

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with Tetryl

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Tetryl
Incubation time:	2 h
Activation:	-
Liver prep. date:	-
Date plated:	27 March 1979
Date counted:	6 April 1979
Plates (batch):	P80341/R40341

TABLE 82 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Tetryl

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	18,330	100	0	0	2.7 (5)	0	1.6 (3)	0	2.2 (4)	0	6.5 (12)
EMS	Dose 8 +ve control 20 mg 80.5mM	26,363	144	11.8 (31)	2.7 (7)	46.2 (122)	3.0 (8)	111.5 (294)	9.9 (26)	71.7 (189)	14.5 (38)	256.8 (677)
Tetryl	Dose 7 31.25µg <0.1mM	5,539	30	0	1.8 (1)	1.8 (1)	1.8 (1)	3.6 (2)	3.6 (2)	7.2 (4)	1.8 (1)	19.8 (11)
	Dose 6 62.5 µg 0.1mM	4,816	26	4.1 (2)	0	4.1 (2)	2.0 (1)	4.1 (2)	4.4 (2)	6.2 (3)	4.1 (2)	24.6 (12)
	Dose 5 125 µg 0.2mM	983	5	10.2 (1)	0	10.2 (1)	0	0	0	20.3 (2)	10.2 (1)	40.7 (4)
	Dose 4 250 µg 0.4mM	388	2	25.8 (1)	0	0	0	0	0	51.5 (2)	25.8 (1)	77.3 (3)
	Dose 3 500 µg 0.9mM	472	3	0	0	0	0	0	0	0	0	0
	Dose 2 1 mg 1.7mM	12	<1	0	0	0	0	0	0	0	0	0
	Dose 1 2 mg 3.5 mM	116 pptn	<1	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate
pptn = precipitation

TABLE 83

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with Tetryl

Project No:	410110
Contractor:	U.S. Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Tetryl
Incubation time:	30 min
Activation:	-
Liver prep. date:	-
Date plated:	24 April 1979
Date counted:	4 May 1979
Plates (batch):	P10441/P43142

TABLE 63 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with Tetryl

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 8 -ve control 400 µl	26,997	100	0.4 (1)	0	0.4 (1)	0	0	0.4 (1)	0.4 (1)	0.4 (1)	1.5 (4)
EMS	Dose 7 +ve control 20 mg 80.5mM	23,562	87	3.8 (9)	3.0 (7)	2.1 (5)	0.8 (2)	7.6 (18)	4.7 (11)	13.2 (31)	6.8 (16)	35.2 (83)
Tetryl	Dose 6 25 µg <0.1mM	29,861	111	0	0	0.7 (2)	0.3 (1)	0.3 (1)	0.3 (1)	0.3 (1)	0	2.0 (6)
	Dose 5 50 µg <0.1mM	29,273	108	0	0.3 (1)	0	0	0.3 (1)	0.3 (1)	1.0 (3)	0.3 (1)	2.0 (6)
	Dose 4 100 µg 0.1mM	24,890	92	0	0	0.4 (1)	0.4 (1)	0.8 (2)	0.8 (2)	1.2 (3)	0	3.6 (9)
	Dose 3 200 µg 0.3mM	22,335	83	0.4 (1)	0	0.4 (1)	0.4 (1)	0.4 (1)	0	1.3 (3)	0.4 (1)	3.1 (7)
	Dose 2 400 µg 0.7mM	13,787	51	0.7 (1)	0.7 (1)	0.7 (1)	0	0.7 (1)	0	1.5 (2)	1.5 (2)	4.4 (6)
	Dose 1 800 µg 1mM	3,684 pptn	14	5.4 (2)	0	2.7 (1)	2.7 (1)	2.7 (1)	5.4 (2)	5.4 (2)	5.4 (2)	24.4 (9)

Figures in parentheses are actual number of aberrants counted

TABLE 84

Saccharomyces cerevisiae D5 Recombination, without activation

Project No.: 410110

Contractor: US Army

Operators: Colin Riach
Jennifer Harvey

Substance: Tetryl

Incubation time: 2 h

Activation: -

Date plated: 25 May 1979

Date counted: 4 June 1979

Plates (batch): M02246

AD-A084 121 INVERESK RESEARCH INTERNATIONAL LTD EDINBURGH (SCOTLAND) F/G 6/5
MUTAGENICITY AND DNA REPAIR POTENTIAL OF 15 CHEMICALS.(U)
JAN 80 D B MCGREGOR DAMD17-78-C-8064

UNCLASSIFIED NL

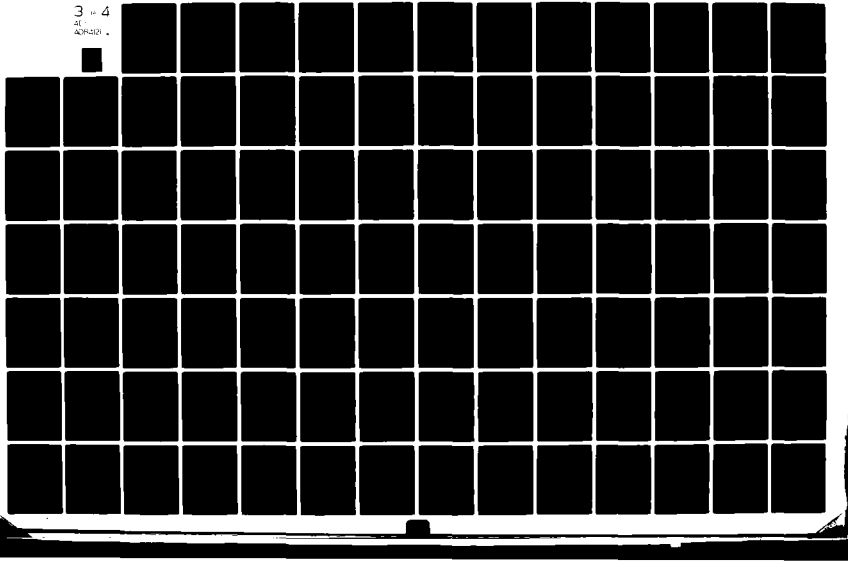


TABLE 84 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Tetryl

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 4 -ve control 200 µl	25,918 (100 plates)	100.0	0	0.8 (2)	0	0	2.3 (6)	4.6 (12)	0.4 (1)	0.8 (2)	8.1 (21)
EMS	Dose 3 +ve control 20 mg 80.5mM	24,593 (100 plates)	94.9	32.9 (81)	15.0 (37)	2.8 (7)	2.4 (6)	180.1 (443)	104.5 (257)	93.1 (229)	48.0 (118)	431.0 (1060)
Tetryl	Dose 2 125 µg 0.2mM	16,326 (200 plates)	31.5	9.2 (15)	2.5 (4)	0.6 (1)	0	8.0 (13)	3.7 (6)	0	11.6 (19)	23.9 (39)
	Dose 1 250 µg 0.4mM	31,390 (600 plates)	20.2	6.4 (20)	1.0 (3)	1.9 (6)	1.3 (4)	8.3 (26)	4.8 (15)	1.0 (3)	7.3 (23)	24.5 (77)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide

EMS = Ethyl methanesulphonate

TABLE 85

S. cerevisiae D5 Recombinogenic Activity with Tetryl, with Metabolic Activation, Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach
	Anne Scott
Substance:	Tetryl
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer Rat
Liver preparation date:	18 July 1979
Date plated	10 August 1979
Date counted	20 August 1979
Plates (Batch)	M26040/R43341

Dilution Factors used to dilute incubation tubes after 18 h incubation

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5 x 10^4
Cyclophosphamide	38.26mg	1 x 10^5
Tetryl	31.3 µg	5 x 10^4
Tetryl	62.5 µg	5 x 10^4
Tetryl	125 µg	1.5 x 10^4
Tetryl	250 µg	3 x 10
Tetryl	500 µg	4 x 10
Tetryl	1 mg	4 x 10
Tetryl	2 mg	1 x 10

TABLE 85 (continued)

S. cerevisiae D5 Recombinogenic Activity
with Tetryl, with Metabolc Activation,
Modified Incubation

Substance	Dose	Colonies counted: Viable count per ml after 18 h incubation	% survival	No. of aberrants/10^4 survivors: Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline	Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	11,893 / 5.9×10^7	100	0.8 (1)	0	0.8 (1)	0	1.7 (2)	0.8 (1)	0.8 (1)	0.8 (1)	5.0 (6)
Cyclo-phos-phamide	Dose 8 +ve control 38.26 mg 68 mM	7,293 / 7.2×10^7	122	9.6 (7)	2.7 (2)	1.4 (1)	0	0	4.1 (3)	0	12.3 (9)	17.8 (13)
Tetryl	Dose 7 31.3 µg 0.05 mM	7,319 / 3.6×10^7	61	0	0	1.4 (1)	0	6.8 (5)	0	1.4 (1)	0	9.6 (7)
	Dose 6 62.5 µg 0.1 mM	9,246 / 4.6×10^7	78	0	1.1 (1)	2.2 (2)	0	5.4 (5)	1.1 (1)	0	1.1 (1)	9.7 (9)
	Dose 5 125 µg 0.2 mM	1,609 / 2.4×10^6	4	0	0	6.2 (1)	0	0	0	6.2 (1)	0	12.4 (2)
	Dose 4 250 µg 0.4 mM	10,493 / 3.1×10^4	<1	1.0 (1)	0	0	0	0	0	0	1.0 (1)	1.0 (1)
	Dose 3 500 µg 0.9 mM	45,047 / 1.8×10^5	<1	0.2 (2)	0	0	0	0	0.1 (1)	0	0.2 (2)	0.6 (3)
	Dose 2 1 mg 1.7 mM	833 / 3.3×10^3	<1 pptn	0	0	0	0	0	12.0 (1)	0	0	12.0 (1)
	Dose 1 2 mg 3.5 mM	3,553 / 3.5×10^3	<1 pptn	2.8 (1)	0	0	2.8 (1)	2.8 (1)	2.8 (1)	0	2.8 (1)	11.3 (4)

Figures in parentheses are actual number of aberrants counted

TABLE 86

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Red phosphorus
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Water	100 µl	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-
Red phosphorus	10.0 mg	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21

TABLE 87

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Date plated: 12 February 1979
Date examined: 13 February 1979

Substance: Red phosphorus
Activation: -
Liver preparation date: -
Batch no. (plates): P30141
Numbering colour Blue

In Suspension		Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Water		50 µl	539	586	723	350	413	456
Ethyl methanesulphonate		10 µl	390	328	516	2	1	3
2-Aminofluorene		6.5 µg	489	517	501	281	282	237
Tube I.D.	Quantity							
G	10.0 µg		679	624	646	205	234	190
F	33.3 µg		718	626	594	359	450	458
E	100.0 µg		493	596	643	558	479	410
D	333.3 µg		608	601	646	346	432	375
C	1.0 mg		533	601	444	579	370	521
B	3.3 mg		432	564	506	492	463	473
A	10.0 mg		724	425	405	421	383	257

TABLE 88

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Date plated: 12 February 1979
Date examined: 13 February 1979

Substance: Red phosphorus
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour: Red

In Suspension	Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Water	50 µl	755	285	1005	268	80	233
Ethyl methanesulphonate	10 µl	378	652	561	8	7	3
2-Aminofluorene	6.5 µg	475	611	453	363	244	424
Tube I.D.	Quantity						
G	10.0 µg	935	825	937	163	214	177
F	33.3 µg	993	1132	982	196	173	206
E	100.0 µg	811	781	793	302	271	204
D	333.3 µg	784	712	868	252	212	299
C	1.0 mg	896	832	919	349	251	303
B	3.3 mg	746	910	845	296	289	238
A	10.0 mg	866	967	1030	295	167	378

TABLE 89

Toxicity Test in Strain TA 98

Substance: Red phosphorus

Project no: 410110

Activation: Arcclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R46549

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 11 December 1978

Blue = without S-9

Date examined: 13 December 1978

Culture batch: A

Toxicity Test	Quantity per Plate	TA 98	
		with S-9	without S-9
Distilled water	100 µl	30	26
Tube I.D.	Quantity		
G	10.0 µg	33	34
F	33.3 µg	31	27
E	100.0 µg	25	22
D	333.3 µg	33	23
C	1.0 mg	34	24
B	3.3 mg	26	27
A	10.0 mg	24	27

Note:- No precipitation but compound present as granules at all doses on plates

TABLE 90

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Red phosphorus

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 11 December 1978

Operator(s): Colin Riach
Batch no. (plates): P88340

Anne Gilroy
Numbering colour(s): Red = with S-9

Date plated: 8 January 1979
Blue = without S-9

Date counted: 10 January 1979
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	10 8 7	8 5 9	33 26 13	21 27 17
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	14 19 24	129 206 182	110 180 137	326 318 424
Red phosphorus	10.0 µg	10 3 6	17 13 10	28 29 21	21 22 20
	33.3 µg	12 15 8	15 15 9	19 25 29	24 33 27
	100.0 µg	11 12 13	13 16 9	30 20 32	24 24 25
	333.3 µg	10 16 12	7 8 17	28 31 26	28 22 23
	1.0 mg	12 23 15	10 15 15	27 23 19	20 29 32
	3.3 mg	17 17 16	11 11 15	29 44 30	34 34 35
	10.0 mg	8 19 17	18 10 8	26 22 22	22 22 33

TABLE 91

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: Red phosphorus

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 16 January 1979

Operator(s): Colin Riach

Batch no. (plates): Red - M85640 Blue P74848

Numbering colour(s): Red = with S-9

Blue = without S-9

Date plated: 17 January 1979

Date counted: 19 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	8 13 9	3 8 5	28 24 36	26 24 31	128 137 135	114 128 127
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	17 23 20	167 179 171	152 135 126	391 429 304	208 190 195	342 317 355
Red phosphorus	10.0 µg	9 9 16	6 14 7	22 22 22	27 26 36	159 119 114	126 138 119
	33.3 µg	4 12 11	9 8 6	25 23 15	39 39 37	108 118 153	130 134 123
	100.0 µg	5 14 18	9 13 7	27 17 23	31 30 36	128 151 124	129 141 125
	333.3 µg	7 14 11	13 6 10	28 22 30	27 38 37	124 131 115	129 127 140
	1.0 mg	11 9 11	13 5 12	23 23 20	34 35 28	148 135 140	141 128 117
	3.3 mg	13 7 9	4 5 9	25 19 19	27 23 30	139 139 142	131 138 125
	10.0 mg	9 9 10	9 10 8	25 29 28	19 31 27	144 145 142	111 132 149

TABLE 92

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: Red phosphorous (P4)

Activation: Aroclor-induced Fischer rat

Date plated: 19 January 1979

Liver preparation date: 16 January 1979

Date counted: 29 January 1979

Batch no. (plates): P 53917

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	89	90	71	83	84
Red phosphorous (P4)	299.5 µg (1.2 mM)	81	91	50	87	181
	29.98 mg (120.6 mM)	79	81	80	68	77
	59.9 mg (241.7 mM)	111	106	91	122	91

Conclusion: Dose range: 48 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	89	111	87	86	100
Red phosphorous (P4)	299.5 µg (1.2 mM)	104	107	92	88	not sampled
	29.9 mg (120.6 mM)	100	97	92	98	84
	59.9 mg (241.7 mM)	98	103	107	85	101

Conclusion: Dose range: 48 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg
Incubation time: 2 h

TABLE 93

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: Red phosphorus

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System N

Date plated: 19 July 1979

Date counted: 25 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2657	2×10^4	5.3×10^7/ml
Red phosphorus	$N3_1$ mg	2675	2×10^4	5.4×10^7/ml
	$N2_{10}$ mg	3275	2×10^4	6.6×10^7/ml
	$N1_{20}$ mg	2229	2×10^4	4.5×10^7/ml

TABLE 94

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Red phosphorus

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach
	Rowan Hastwell
Substance:	Red Phosphorus
Incubation time:	2 h
Activation:	-
Liver preparation date:	-
Date plated:	6 March 1979
Date counted:	16 March 1979
Plates (Batch):	P71107/R50241

TABLE 93 (continued)

Conclusion:-	Doses for full test	Dilution factor
	N7 312.5 µg	5×10^4
	N6 625 µg	5×10^4
	N5 1.25 mg	5×10^4
	N4 2.5 mg	5×10^4
	N3 5 mg	5×10^4
	N2 10 mg	5×10^4
	N1 20 mg	5×10^4

TABLE 94 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity
without activation, with Red phosphorus

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	16227	100	1.2 (2)	0	1.8 (3)	0.6 (1)	(1)	0	12.3 (20)	1.2 (2)	15.9 (27)
EMS	Dose 8 +ve control 20 mg 80.5mM	14943	92.1	38.8 (58)	19.4 (29)	31.5 (47)	7.4 (11)	58.2 (87)	62.2 (93)	170.6 (255)	58.2 (87)	388.1 (580)
Red phosphorus (P_4)	Dose 7 1 mg 4.0mM	15323	94.4	0.7 (1)	1.3 (2)	0	0	2.0 (3)	1.3 (2)	3.3 (5)	2.0 (3)	8.6 (13)
	Dose 6 2 mg 8.1mM	16119	99.3	1.2 (2)	1.2 (2)	0.6 (1)	0.6 (1)	1.2 (2)	1.2 (2)	1.2 (2)	2.4 (4)	7.2 (12)
	Dose 5 4 mg 16.1mM	15836	97.6	0.6 (1)	0.6 (1)	2.5 (4)	0.6 (1)	1.3 (2)	1.3 (2)	2.5 (4)	1.2 (2)	9.4 (15)
	Dose 4 8 mg 32.3mM	13455	82.9	1.5 (2)	0	0.7 (1)	1.5 (2)	0.7 (1)	0.7 (1)	2.2 (3)	1.5 (2)	7.3 (10)
	Dose 3 16 mg 64.6mM	15960	98.4	0.6 (1)	0.6 (1)	3.1 (5)	0	1.2 (2)	1.9 (3)	2.5 (4)	1.2 (2)	7.6 (16)
	Dose 2 32 mg 129.1mM	14668	90.4	0.7 (1)	0	0.7 (1)	0.7 (1)	1.4 (2)	0.7 (1)	3.4 (5)	0.7 (1)	7.6 (11)
	Dose 1 48 mg 193.7mM	15193	93.6	0	0.7 (1)	0.7 (1)	0.7 (1)	0.7 (1)	2.0 (3)	2.0 (3)	0.7 (1)	6.8 (10)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate NB = Red phosphorus not in solution at any dose

TABLE 95

S. cerevisiae D5 Recombinogenic Activity with Red phosphorus, with Metabolic activation Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Jennifer Harvey Christopher Corden
Substance:	Red phosphorus
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	15 August 1979
Date plated:	7 September 1979
Date counted:	17 September 1979
Plates (Batch):	B10841

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
Red phosphorus	312.5 µg	5×10^4
Red phosphorus	625 µg	5×10^4
Red phosphorus	1.25 mg	5×10^4
Red phosphorus	2.5 mg	5×10^4
Red phosphorus	5.0 mg	5×10^4
Red phosphorus	10.0 mg	5×10^4
Red phosphorus	20.0 mg	5×10^4

TABLE 95 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with Red phosphorus, with metabolic activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	1312 / 6.6×10^6	100	0	0	7.6 (1)	7.6 (1)	7.6 (1)	0	0	0	22.9 (3)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	10265 / 1.0×10^8	1515	1.0 (1)	1.9 (2)	0	1.0 (1)	0	1.9 (2)	1.0 (1)	2.9 (3)	6.8 (7)
Red phos-phorus	Dose 7 312.5 µg	8912 / 4.5×10^7	682	0	0	0	1.1 (1)	2.2 (2)	2.2 (2)	0	0	5.6 (5)
	Dose 6 625 µg	9393 / 4.7×10^7	712	0	0	2.1 (2)	1.1 (1)	0	1.1 (1)	0	0	4.3 (4)
	Dose 5 1.25 mg	7895 / 3.9×10^7	591	0	1.3 (1)	1.3 (1)	5.1 (4)	6.3 (5)	0	0	1.3 (1)	13.9 (11)
	Dose 4 2.5 mg	7868 / 3.9×10^7	591	0	0	0	0	2.5 (2)	3.8 (3)	0	0	6.4 (5)
	Dose 3 5.0 mg	7224 / 3.6×10^7	545	1.4 (1)	0	1.4 (1)	0	4.2 (3)	0	0	1.4 (1)	6.9 (5)
	Dose 2 10.0 mg	8032 / 4.0×10^7	606	0	1.2 (1)	3.7 (3)	0	0	0	0	1.2 (1)	5.0 (4)
	Dose 1 20.0 mg	7893 / 3.9×10^7	591	1.3 (1)	0	5.1 (4)	1.3 (1)	3.8 (3)	6.3 (5)	1.3 (1)	1.3 (1)	19.0 (15)

Figures in parentheses are actual number of aberrants counted

TABLE 96

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Nitroguanidine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Nitroguanidine	10.0 mg	with S-9	16 n	16 n	16 n	-	17 n	17 n
		without S-9	17 n	17 n	17 n	17 n	17 n	17 n

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
n = non specific killing

TABLE 97

Toxicity Test in Strain TA 98

Substance: Nitroguanidine

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 25 May 1978

Operator(s): Rowan Hastwell
Batch no. (plates): R46549

Anne Gilroy
Numbering colour(s): Red = with S-9

Date plated: 4 December 1978
Blue = without S-9

Date counted: 6 December 1978
Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
Nitroguanidine	10.0 µg	26	29
	33.3 µg	32	28
	100.0 µg	41	18
	333.3 µg	33	23
	1.0 mg	31	26
	3.3 mg	29	33
	10.0 mg	26	27

TABLE 98

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Nitroguanidine

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): P13844

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 20 December 1978

Blue = without S-9

Date counted: 22 December 1978

Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	19 8 8	16 21 15	11 15 26	17 16 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	48 26 20	12 14 13	232 347 195	306 366 290
Nitroguanidine	10.0 µg	18 16 8	9 13 12	30 20 26	26 17 32
	33.3 µg	18 5 14	7 8 13	17 15 20	31 28 17
	100.0 µg	12 8 4	13 6 contam	26 36 21	15 20 28
	333.3 µg	9 13 7	6 7 6	28 28 12	21 18 27
	1.0 mg	13 9 9	8 14 12	19 19 26	29 24 26
	3.3mg	19 8 9	8 11 8	19 16 16	23 21 21
	10.0mg	10 7 7	11 6 9	14 16 15	25 23 25

contɯm = contamination

TABLE 99

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 22 January 1979
Date counted: 24 January 1979

Substance: Nitroguanidine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P67542
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: D

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	11 6 6	6 8 5	25 20 24	26 25 36	135 145 131	138 129 115
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	20 9 18	643 578 623	88 103 80	663 605 574	214 269 2[illegible]6	374 356 404
Nitroguanidine	10.0 µg	14 9 5	12 13 11	29 30 23	34 27 23	117 118 118	146 124 141
	33.3 µg	9 4 12	12 12 13	22 25 23	16 19 33	116 89 117	133 147 151
	100.0 µg	14 12 11	4 16 17	35 37 contam	20 18 26	133 136 146	86 122 137
	333.3 µg	7 9 11	6 16 11	26 27 49	25 29 28	140 145 146	124 162 136
	1.0 mg	12 14 16	10 12 20	36 38 27	17 34 31	142 119 114	108 135 135
	3.3 mg	3 13 9	9 8 17	22 29 25	28 22 34	120 141 130	129 134 201
	10.0 mg	9 8 6	11 9 9	17 40 37	44 34 37	131 146 107	156 139 138

contam = contamination

TABLE 100

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410.10

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Nitroguanidine

Activation: Aroclor-induced Fischer rat

Date plated: 23 January 1979

Liver preparation date: 16 January 1979

Date counted: 29 January 1979

Batch no. (plates): R 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	96	80	84	82	82
Nitro-guanidine	10 mg (48.0 mM)	96	90	102	89	87
	22.6 mg (108.5 mM)	86	83	91	77	72
	45.3 mg (217.6 mM)	95	88	93	75	52

Conclusion: Dose range: Saturation, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	71	468	62	69	56
Nitro-guanidine	10 mg (48.0 mM)	93	559	102	90	106
	22.6 mg (108.5 mM)	93	514	71	87	69
	45.3 mg (217.6 mM)	101	68	54	63	59

Conclusion: Dose range: Saturation, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg, 1 mg
Incubation time: 2 h

TABLE 101

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Project no: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Jennifer Harvey
Date plated: 19 July 1979
Date counted: 25 July 1979

Substance: Nitroguanidine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): BO1441
Numbering System P

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2657	2×10^4	5.3×10^7/ml
Nitroguanidine	P3 2 mg	2433	2×10^4	4.9×10^7/ml
	P2 10 mg	2513	2×10^4	5.0×10^7/ml
	P1 20 mg	2420	2×10^4	4.8×10^7/ml

TABLE 101 (continued)

Conclusion:-	Dose for full test	Dilution factor
	P7 500 µg	5×10^4
	P6 1 mg	5×10^4
	P5 2 mg	5×10^4
	P4 4 mg	5×10^4
	P3 8 mg	5×10^4
	P2 16 mg	5×10^4
	P1 Saturation	5×10^4

TABLE 102

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with nitroguanidine

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Anne Scott Jennifer Harvey
Substance:	Nitroguanidine
Incubation time:	2 h
Activation:	-
Liver prep. date:	-
Date plated:	20 March 1979
Date counted:	30 March 1979
Plates (batch):	M82041/P80341

TABLE 102 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with nitroguanidine

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	24,507	100	0.4 (1)	0.8 (2)	2.0 (5)	2.4 (6)	1.2 (3)	0.8 (2)	1.6 (4)	1.2 (3)	9.2 (23)
EMS	Dose 8 +ve control 20 mg 80.5mM	25,624	104	45.2 (116)	57.3 (147)	3.9 (23)	5.9 (15)	131.5 (337)	60.5 (155)	49.6 (127)	102.5 (263)	358.9 (920)
Nitro-guanidine	Dose 7 1 mg 5mM	28,101	115	0	0.7 (2)	1.4 (4)	3.6 (1)	1.8 (5)	3.6 (1)	1.4 (4)	0.7 (2)	12.5 (17)
	Dose 6 2 mg 10mM	27,984	114	0	0	1.7 (5)	1.1 (3)	3.6 (10)	1.4 (4)	2.5 (7)	0	10.3 (29)
	Dose 5 4 mg 19mM	21,870	89	1.8 (4)	0.4 (2)	3.7 (8)	0.5 (1)	0.5 (1)	0.9 (2)	2.3 (5)	2.7 (6)	10.6 (23)
	Dose 4 8 mg 38mM	19,545	80	0.5 (1)	0.5 (1)	1.5 (3)	1.0 (2)	2.0 (4)	2.0 (4)	4.1 (8)	1.0 (2)	11.6 (23)
	Dose 3 16 mg 77mM	22,024 pptn	90	0.9 (2)	0.9 (2)	1.8 (4)	0.9 (2)	1.4 (3)	0.9 (2)	1.4 (3)	1.8 (4)	8.2 (18)
	Dose 2 32 mg 154mM	19,053 pptn	78	1.0 (2)	1.0 (2)	1.6 (3)	0.5 (1)	1.6 (3)	0.5 (1)	1.6 (3)	2.0 (4)	6.8 (15)
	Dose 1 54.1mg 260mM	23,673 pptn	97	0.4 (1)	0	0.4 (1)	1.3 (3)	2.5 (6)	0.8 (2)	1.3 (3)	0.4 (1)	6.7 (16)

Figures in parentheses are actual number of aberrants counted DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate
pptn = precipitation

TABLE 103

S. cerevisiae D5 Recombinogenic Activity with Nitroguanidine, with Metabolic activation Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Nitroguanidine
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	20 September 1979
Date plated:	21 September 1979
Date counted:	1 October 1979
Plates (Batch):	B10841

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
Nitroguanidine	375 µg	5×10^4
Nitroguanidine	750 µg	5×10^4
Nitroguanidine	1.5 mg	5×10^4
Nitroguanidine	3.0 mg	5×10^4
Nitroguanidine	6.0 mg	5×10^4
Nitroguanidine	12.0 mg	5×10^4
Nitroguanidine	24.0 mg	5×10^4

TABLE 103 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with Nitroguanidine, with metabolic activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Fed	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	16287 / 8.1×10^7	100	1.2 (2)	0.6 (1)	1.2 (2)	0	0.6 (1)	0.6 (1)	1.2 (2)	1.8 (3)	5.5 (9)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	10035 / 1×10^8	123	4.0 (4)	1.0 (1)	1.0 (1)	0	4.0 (4)	1.0 (1)	8.0 (8)	5.0 (5)	19.0 (19)
Nitro-guanidine	Dose 7 375 mg	13521 / 6.8×10^7	84	0.7 (1)	0	2.2 (3)	1.5 (2)	0.7 (1)	0	0.7 (1)	0.7 (1)	5.9 (8)
	Dose 6 750 µg	14984 / 7.5×10^7	93	0.7 (1)	0	0	0	0	0	1.3 (2)	0.7 (1)	2.0 (3)
	Dose 5 1.5 mg	14272 / 7.1×10^7	88	0	0.7 (1)	2.1 (3)	0.7 (1)	2.1 (2)	0	0.7 (1)	0.7 (1)	5.6 (8)
	Dose 4 3.0 mg	14211 / 7.1×10^7	88	0.7 (1)	0	2.1 (3)	0.7 (1)	0	0.7 (1)	2.8 (4)	0.7 (1)	7.0 (10)
	Dose 3 6.0 mg	12917 / 6.5×10^7	80	0.8 (1)	0	4.6 (6)	0.8 (1)	0	0	1.5 (2)	0.8 (1)	7.7 (10)
	Dose 2 12.0 mg	13684 / 6.8×10^7	84	0	0	2.2 (3)	0	2.9 (4)	0	1.5 (2)	0	6.6 (9)
	Dose 1 24.0 mg	15516 / 7.6×10^7	94	0.6 (1)	0	2.6 (4)	1.3 (2)	1.3 (2)	1.3 (2)	0.6 (1)	0.6 (1)	7.7 (12)

Figures in parentheses are actual number of aberrants counted

TABLE 104

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: N-nitrosodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
N-nitro-sodiphenylamine	10.0 mg pptn	with S-9	-	-	-	-	-	-
		without S-9	-	-	-	-	-	-

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
pptn = precipitation

TABLE 105

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Rowan Hastwell

Date plated: 12 February 1979
Date examined: 13 February 1979

Substance: N-nitrosodiphenylamine
Activation: -
Liver preparation date: -
Batch no. (plates): P30141
Numbering colour Blue

In Suspension		Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide		50 µl	317	469	230	296	448	272
Ethyl methanesulphonate		10 µl	182	147	150	2	5	1
2-Aminofluorene		6.5 µg	330	522	439	273	203	241
Tube I.D.	Quantity							
G	10.0 µg		370	322	529	312	152	192
F	33.3 µg		480	430	403	304	359	311
E	100.0 µg pptn		260	345	729	417	186	317
D	333.3 µg pptn		662	340	228	362	392	190
C	1.0 mg pptn		433	384	449	381	146	85
B	3.3 mg pptn		138	No bacteria	239	160	114	238
A	10.0 mg pptn		106	108	143	169	399	329

pptn = precipitation

TABLE 106

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Colin Riach
Date plated: 28 March 1979
Date examined: 29 March 1979

Substance: N-nitrosodiphenylamine
Activation: -
Liver preparation date: -
Batch no. (plates): P30141
Numbering colour Blue

In Suspension	Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide	50 µl	1652	1700	1659	915	748	849
Ethyl methanesulphonate	10 µl	1622	1491	1392	11	13	5
2-Aminofluorene	6.5 µg	1658	1583	1658	685	786	732
Tube I.D.	Quantity						
4	pptn 1.25 mg	1452	1485	1586	925	958	865
3	pptn 2.5 mg	1404	1546	1607	815	840	798
2	pptn 5.0 mg	1635	1620	1521	801	918	653
1	pptn 10.0 mg	1609	1692	1549	620	724	543

pptn = precipitation

TABLE 107

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Rowan Hastwell

Date plated: 12 February 1979
Date examined: 13 February 1979

Substance: N-nitrosodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour Red

In Suspension	Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide	50 µl	747	547	608	572	857	584
Ethyl methanesulphonate	10 µl	472	578	199	11	4	4
2-Aminofluorene	6.5 µg	572	580	585	390	386	525
Tube I.D.	Quantity						
G	10.0 µg	535	684	654	366	453	356
F	33.3 µg	562	501	675	359	318	505
E	100.0 µg	578	583	301	540	391	438
D	333.3 µg	453	301	560	255	411	436
C	1.0 mg	270	340	475	281	473	191
B	3.3 mg	603	437	395	293	461	405
A	10.0 mg	575	734	754	4*	2*	3*

* bacteria not incorporated adequately

TABLE 108

DNA Repair Test in Suspension

Project No: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 28 March 1979
Date examined: 29 March 1979

Substance: N-nitrosodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 14 March 1979
Batch no. (plates): P30141
Numbering colour Red

In Suspension		Quantity	pol A^+ (100 µl)			pol A^- (100 µl)		
(Solvent) Dimethylsulphoxide		50 µl	1865	1793	1863	993	975	1027
Ethyl methanesulphonate		10 µl	1775	1721	1806	67	72	127
2-Aminofluorene		6.5 µg	1719	1847	1911	981	1040	981
Tube I.D.	Quantity							
4	pptn 1.25 mg		1775	1799	1833	1077	1040	1091
3	pptn 2.5 mg		1890	1894	1914	1092	1012	1046
2	pptn 5.0 mg		1875	1941	1832	1104	1082	1129
1	pptn 10.0 mg		1973	1951	1948	1120	989	1110

pptn = precipitation

TABLE 109

Toxicity Test in Strain TA 98

Substance: N-nitrosodiphenylamine

Project no: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Anne Gilroy
Date plated: 4 December 1978
Date counted: 6 December 1978

Activation: Aroclor-induced Fischer rat
Liver preparation date: 25 May 1978
Batch no. (plates): R54549
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	29	24
N-nitrosodiphenylamine	10.0 µg	35	21
	33.3 µg	36	20
	100.0 µg	40	31
	333.3 µg	24	26
	1.0 mg pptn	40	23
	3.3 mg pptn	19	34
	10.0 mg pptn	9	*

pptn = precipitation
*precipitation too dense to enable accurate counting

TABLE 110

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: N-nitrosodiphenylamine

Project no: 410110
Activation: Aroclor-induced Fischer rat

Contractor: US Army
Liver preparation date: 11 December 1978

Operator(s): Colin Riach
Batch no. (plates): P13844

Anne Gilroy
Numbering colour(s): Red = with S-9

Date plated: 20 December 1978
Blue = without S-9

Date counted: 22 December 1978
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	19 8 8	16 21 15	11 15 26	17 16 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	48 26 20	12 14 13	232 347 195	306 366 290
N-nitroso-diphenylamine	10.0 µg	9 11 2	6 6 9	17 13 12	14 15 21
	33.3 µg	13 8 13	10 11 12	20 20 17	13 16 13
	100.0 µg	9 12 6	6 6 13	15 19 21	10 19 19
	333.3 µg pptn	13 7 7	4 14 8	24 26 19	18 23 21
	1.0 mg pptn	3 2 3	9 12 8	9 9 8	6 12 12
	3.3 mg pptn	3 8 4	5 5 4	2 1 4	4 3 6
	10.0 mg pptn	VTL VTL VTL	VTL VTL VTL	STL *STL STL	STL *STL STL

pptn = precipitation:
*precipitation too dense to enable accurate counting:
STL = slightly thin lawn: VTL = very thin lawn

TABLE 111

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 22 January 1979
Date counted: 24 January 1979

Substance: N-nitrosodiphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P67542
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: C on 19 January
D on 22 January 1979

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	11 6 6	6 8 5	25 20 24	26 25 36	135 145 131	138 129 115
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	20 9 18	643 578 623	88 103 80	663 605 574	214 269 246	374 356 404
N-nitro-sodiphenylamine	10.0 µg	9 8 13	3 8 13	20 28 19	26 20 35	142 174 175	131 128 144
	33.3 µg	14 contam 14	14 11 7	22 11 26	25 28 15	160 133 148	136 161 124
	100.0 µg	12 8 13	15 15 9	24 27 14	25 26 30	118 145 152	130 159 167
	333.3 µg pptn	6 7 13	6 14 14	18 18 17	33 34 29	136 157 144	157 147 125
	1.0 mg pptn	4 3 0	7 4 9	30 26 23	33 31 29	157 145 177	149 156 185
	3.3 mg	2TL 2TL 0TL	6TL 2TL 2TL	4TL 2TL 6TL	7TL 2TL 11TL	149 145 173	138 168 159
	10.0 mg	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	VTL VTL VTL	STL *STL STL	STL *STL STL

* = precipitation too dense to enable accurate counting
STL = slightly thin lawn
TL = thin lawn
VTL = very thin lawn
contam = contamination

TABLE 112

Saccharomyces cerevisiae - Toxicity Test

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: N-nitrosodiphenylamine

Activation: Without activation

Date plated: 12 December 1978

Liver preparation date: -

Date counted: 21 December 1978

Numbering colour: Black

Substance	Quantity per Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Total	% Survival
Dimethyl-sulphoxide	200 µl	63	131	90	98	117	499	100
N-nitroso-diphenyl-amine	2.0 mg	88	101	109	91	108	497	100
	4.0 mg	96	79	92	78	92	437	87
	8.0 mg	110	92	90	104	99	395	79
	16.0 mg	87	109	84	76	110	466	93
	32.0 mg	112	130	99	105	100	546	100
	64.0 mg	114	132	96	147	127	616	100
	118.4 mg	133	171	187	145	157	793	100

TABLE 113

Saccharomyces cerevisiae - Toxicity Test

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: N-nitrosodiphenylamine

Activation: Aroclor-induced Fischer rat

Date plated: 12 December 1978

Liver preparation date: 11 December 1978

Date counted: 21 December 1978

Numbering colour: Blue

Substance	Quantity per Plate	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Total	% Survival
Dimethyl-sulphoxide	200 µl	88	108	98	127	131	552	100
N-nitroso-diphenyl-amine	2.0 mg	73	92	130	84	117	496	89
	4.0 mg	107	99	78	74	61	419	75
	8.0 mg	148	133	159	105	153	698	100
	16.0 mg	109	141	105	108	84	547	100
	32.0 mg	163	181	125	145	114	628	100
	64.0 mg	184	155	187	160	136	822	100
	118.4 mg	157	156	111	112	168	704	100

TABLE 114

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: N-nitrosodiphenylamine

Activation: Aroclor-induced Fischer rat

Date plated: 16 January 1979

Liver preparation date: 16 January 1979

Date counted: 22 January 1979

Batch no. (plates): R 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	152	128	104	83	108
N-nitroso-diphenylamine	333.3 µg (0.8 mM)	130	121	101	71	98
	52.7 mg (132.9 mM)	85	115	87	92	96
	105.4 mg (265.8 mM)	133	122	91	105	90

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	139	112	95	93	83
N-nitroso-diphenylamine	333.3 µg (0.8 mM)	114	113	87	80	110
	52.7 mg (132.9 mM)	107	103	97	71	124
	105.4 mg (265.8 mM)	130	89	111	84	94

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 115

Toxicity Test in S. cerevisiae D5 - 18 h Incubtion with Metabolic Activation

Substance: N-nitrosodiphenylamine

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System D

Date plated: 19 July 1979

Date counted: 25 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2657	2×10^4	5.3×10^7/ml
N-nitroso-diphenylamine	$D3_1$ mg pptn	1457	2×10^4	2.9×10^7/ml
	$D2_2$ 0 mg pptn	2258	2×10^4	4.5×10^7/ml
	$D1_4$ 0 mg pptn	2495	2×10^4	5.0×10^7/ml

pptn = precipitation

TABLE 115 (continued)

Conclusion:-	Doses for full test	Dilution factor
	D7 1 mg	5×10^4
	D6 2 mg	5×10^4
	D5 4 mg	5×10^4
	D4 8 mg	5×10^4
	D3 16 mg	5×10^4
	D2 32 mg	5×10^4
	D1 Saturation	5×10^4

TABLE 116

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with *N*-nitrosodiphenylamine

Project No:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Anne Gilroy
Substance:	*N*-nitrosodiphenylamine
Incubation time:	2 h
Activation:	-
Liver preparation date:	-
Date plated:	13 March 1979
Date counted:	23 March 1979
Plates (Batch):	M70218

TABLE 115 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity without activation, with N-nitrosodiphenylamine

Test chem.	Dose	No. cells plated	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	13,084	100	0	0.8 (1)	0.8 (1)	2.2 (3)	0.8 (1)	0.8 (1)	6.8 (9)	0.8 (1)	12.2 (16)
EMS	Dose 8 +ve control 20 mg 80.5mM	14,932	114.1	44.9 (67)	17.4 (26)	19.4 (29)	6.0 (9)	77.0 (115)	83.0 (124)	172.1 (257)	62.3 (93)	419.8 (627)
N-nitroso-diphenyl-amine	Dose 7 2 mg 5.0mM	10,729 pptn	82.0	0	0	2.8 (3)	0.9 (1)	0	1.9 (2)	3.7 (4)	0	9.3 (10)
5.0 mM 10.1 mM	Dose 6 4 mg 10.1mM	14,719 pptn	112.5	0	1.4 (2)	0.7 (1)	1.4 (2)	2.0 (3)	0.7 (1)	2.7 (4)	1.4 (2)	8.9 (13)
	Dose 5 8 mg 20.2mM	13,343 pptn	102.0	2.2 (3)	0	1.1 (1)	1.1 (1)	0	0	12.4 (11)	2.2 (3)	16.8 (16)
	Dose 4 16 mg 40.4mM	13,633 pptn	104.2	0	0	0	1.5 (2)	2.2 (3)	2.9 (4)	5.9 (8)	0	12.5 (17)
	Dose 3 32 mg 80.7mM	13,807 pptn	105.5	0.7 (1)	2.2 (3)	0.7 (1)	2.9 (4)	0.7 (1)	0.7 (1)	2.9 (4)	2.9 (4)	10.8 (15)
	Dose 2 64 mg 161.4mM	15,973 pptn	122.1	0.6 (1)	0	1.9 (3)	0.6 (1)	0.6 (1)	1.3 (2)	5.6 (9)	0.6 (1)	10.6 (17)
	Dose 1 130 mg 327.9mM	18,894 pptn	144.4	0	1.1 (2)	1.6 (3)	2.1 (4)	1.6 (3)	1.6 (3)	4.2 (8)	1.1 (2)	12.2 (23)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate pptn = precipitation

TABLE 117

Saccharomyces cerevisiae D5 Recombinogenic Activity
with activation and *N*-nitrosodiphenylamine

Project No:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Colin Riach
Substance:	*N*-nitrosodiphenylamine
Incubation time:	2 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	16 January 1979
Date plated:	13 February 1979
Date counted:	23 February 1979
Plates (Batch):	R21040

TABLE 117 (continued)

Saccharomyces cerevisiae D5 Recombinogenic activity with activation and N-nitrosodiphenylamine

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	31,982	100	0	0.3 (1)	0.6 (2)	0	0	0	0.6 (2)	0.3 (1)	1.5 (5)
DMN	Dose 8 +ve control 20 mg 135.0mM	35,140	109.8	0.3 (1)	0.3 (1)	1.1 (4)	0.3 (1)	0.3 (1)	0.9 (3)	0.3 (1)	0.6 (2)	3.5 (12)
N-nitroso-diphenylamine	Dose 7 2 mg 5.0mM	33,021 pptn	103.2	0	0	0.3 (1)	0.9 (3)	0	0	1.2 (4)	0	2.4 (8)
	Dose 6 4 mg 10.1mM	32,565 pptn	101.8	0	0	0	0	0.3 (1)	0	0.6 (2)	0	0.9 (3)
	Dose 5 8 mg 20.2mM	30,935 pptn	96.7	0	1.0 (3)	1.0 (3)	0.6 (2)	0.6 (2)	0.3 (1)	1.3 (4)	1.0 (3)	4.8 (15)
	Dose 4 16 mg 40.4mM	33,175 pptn	103.7	0.9 (3)	0	0	1.8 (6)	0	0.6 (2)	0	0.9 (3)	3.3 (11)
	Dose 3 32 mg 80.7mM	31,771 pptn	99.3	0.3 (1)	0.3 (1)	1.3 (4)	0	0	0	0.9 (3)	0.6 (2)	2.8 (9)
	Dose 2 64 mg 161.4mM	28,135 pptn	87.9	0.4 (1)	0.4 (1)	1.8 (5)	0.7 (2)	1.1 (3)	0.4 (1)	1.4 (4)	0.8 (2)	6.2 (17)
	Dose 1 128.7mg 324.6mM	24,225 pptn	75.7	0	0.4 (1)	0.8 (2)	0	1.2 (3)	0.4 (1)	0.4 (1)	0.4 (1)	3.2 (8)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide DMN = Dimethyl nitrosamine pptn = precipitation

TABLE 118

S. cerevisiae D5 Recombinogenic Activity with N-nitroso-diphenylamine, with Metabolic activation
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Jennifer Harvey Christopher Corden
Substance:	N-nitrosodiphenylamine
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	29 August 1979
Date plated:	13 September 1979
Date counted:	21 September 1979
Plates (Batch):	B10841

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
N-nitrosodiphenylamine	1.0 mg	5×10^4
N-nitrosodiphenylamine	2.0 mg	5×10^4
N-nitrosodiphenylamine	4.0 mg	5×10^4
N-nitrosodiphenylamine	8.0 mg	5×10^4
N-nitrosodiphenylamine	16.0 mg	5×10^4
N-nitrosodiphenylamine	32.0 mg	5×10^4
N-nitrosodiphenylamine	74.8 mg	5×10^4

TABLE 118 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with N-nitrosodiphenylamine with metabolic activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	12837 / 6.4×10^7	100	0.8 (1)	1.6 (2)	0	0	0	0.8 (1)	1.6 (2)	2.3 (3)	4.7 (6)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	10148 / 1×10^8	156	1.0 (1)	3.0 (3)	0	0	1.0 (1)	1.0 (1)	0	3.9 (4)	.9 (6)
N-nitroso-diphenyl amine	Dose 7 1.0 mg pptn	6288 / 3.1×10^7	48	0	0	3.2 (2)	0	0	1.6 (1)	0	0	4.8 (3)
	Dose 6 2.0 mg pptn	8399 / 4.2×10^7	66	1.2 (1)	0	2.4 (2)	0	0	0	0	1.2 (1)	3.6 (3)
	Dose 5 4.0 mg pptn	9522 / 4.8×10^7	75	0	1.1 (1)	0	0	2.1 (2)	1.1 (1)	1.1 (1)	1.1 (1)	5.3 (5)
	Dose 4 8.0 mg pptn	9055 / 4.5×10^7	70	0	0	3.3 (3)	1.1 (1)	1.1 (1)	0	2.2 (2)	0	7.7 (7)
	Dose 3 16.0 mg pptn	9489 / 4.7×10^7	73	0	0	4.2 (1)	1.1 (1)	3.2 (3)	1.1 (1)	1.1 (1)	0	10.5 (10)
	Dose 2 32.0 mg pptn	10229 / 5.1×10^7	80	1.0 (1)	0	4.9 (5)	1.0 (1)	1.0 (1)	0	2.0 (2)	1.0 (1)	9.8 (10)
	Dose 1 74.8 mg pptn	10189 / 5.1×10^7	80	0	1.0 (1)	2.0 (2)	2.0 (2)	1.0 (1)	0	0	1.0 (1)	5.9 (6)

Figures in parentheses are actual number of aberrants counted
pptn = precipitation

TABLE 119

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Diphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Diphenylamine	10.0 mg pptn	with S-9	16 n	16 n	16 n	16 n	16 n	16 n
		without S-9	16 n	16 n	16 n	16 n	16 n	16 n

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
pptn = precipitation
n = non specific killing

TABLE 120

Toxicity Test in Strain TA 98

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 11 December 1978
Date counted: 13 December 1978

Substance: Diphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): R46549
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	23	22
Diphenylamine	10.0 µg	31	21
	33.3 µg	37	23
	100.0 µg	28	28 STL
	333.3 µg	24 TL	33 TL
	1.0 mg pptn	NL	NL
	3.3 mg pptn	NL	NL
	10.0 mg pptn	NL	NL

pptn = precipitation
STL = slightly thin lawn
TL = thin lawn
NL = no lawn i.e. complete killing

TABLE 121

Salmonella Plate Test in Strains TA 1535 and TA 98

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 20 December 1978
Date counted: 22 December 1978

Substance: Diphenylamine
Activation: Aroclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): P13844
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	19 8 8	16 21 15	11 15 26	17 16 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	48 26 20	12 14 13	232 347 195	306 366 290
Diphenylamine	1.0 µg	11 7 6	8 10 8	21 30 24	19 27 24
	3.3 µg	8 8 9	9 9 8	30 16 15	19 15 14
	10.0 µg	11 11 12	5 4 8	23 15 15	22 13 21
	33.3 µg	4 11 7	7 7 10	21 15 19	25 16 20
	100.0 µg	14 11 4	8 6 14	15 21 18	19 22 20
	333.3 µg pptn	VTL VTL VTL	VTL VTL VTL	10TL 12TL 11TL	5TL 3TL 3TL
	1.0 mg pptn	NL NL NL	NL NL NL	NL NL NL	NL NL NL

pptn = precipitation: TL = thin lawn:
VTL = very thin lawn: NL = no lawn i.e. complete killing

TABLE 122

Salmonella Plate Test in Strains TA 1537, [illegible]

Substance: Diphenylamine

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 16 January 1979

Operator(s): Colin Riach

Batch no. (plates): P90142

Numbering colour: Red = with S-9

Blue = without S-9

Date plated: 24 January 1979

Date counted: 26 January 1979

Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	2 6 4	11 13 8	26 contam 34	35 34 29	120 149 115	129 129 139
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	17 19 12	1099 855 991	268 305 242	534 524 621	264 242 258	260 281 300
Diphenylamine	1.0 µg	6 13 8	9 7 4	39 39 contam	23 26 28	130 148 102	126 124 86
	3.3 µg	12 3 12	2 11 7	36 36 34	19 19 22	173 131 129	117 120 134
	10.0 µg	11 9 6	7 11 14	22 33 29	23 25 30	141 145 117	139 123 134
	33.3 µg	7 12 5	14 15 4	29 37 34	31 31 22	173 144 157	153 161 137
	100.0 µg	5 13 7	11 7 14	20 31 22	22 34 31	157 150 157	128 111 126
	333.3 µg pptn	4TL 6TL 4TL	7TL 14TL 5TL	33TL 25TL 35TL	21STL 25STL 24STL	147STL 158STL 151STL	125STL 129STL 129STL
	1.0 [illegible] pptn	NL NL NL	NL NL NL	NL NL NL	NL NL NL	VTL VTL VTL	NL NL NL

STL = slightly thin lawn
TL = thin lawn
VTL = very thin lawn
NL = complete killing
contam = contamination
pptn = precipitation

TABLE 123

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 4110110

Contractor: US Army

Operator(s): Rowan Hastwell
Colin Riach

Substance: Diphenylamine

Activation: Aroclor-induced Fischer rat

Date plated: 23 January 1979

Liver preparation date: 16 January 1979

Date counted: 29 January 1979

Batch no. (plates): R 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	65	72	86	84	59
Diphenylamine	500 µg (1.4 mM)	0	<1	<1	0	<1
	43.0 mg (127.0 mM)	0	0	<1	0	0
	86.0 mg (254.1 mM)	1	1	<1	<1	<1

Conclusion: Dose range: 500 µg, 250 µg, 125 µg, 62.5 µg, 31.25 µg, 15.62 µg, 7.81 µg
Incubation time: 30 min

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	56	73	67	69	94
Diphenylamine	500 µg (1.4 mM)	<1	2	<1	0	0
	43.0 mg (127.0 mM)	0	0	<1	0	0
	86.0 mg (254.1 mM)	0	<1	<1	<1	<1

Conclusion: Dose range: 500 µg, 250 µg, 125 µg, 62.5 µg, 31.25 µg, 15.62 µg, 7.81 µg
Incubation time: 30 min

TABLE 124

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: Diphenylamine

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System T

Date plated: 20 July 1979

Date counted: 26 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2410	2×10^4	4.8×10^7/ml
Diphenylamine	T^7 15.63 µg	2769	2×10^4	5.5×10^7/ml
	T^6 31.25 µg	3362	2×10^4	6.7×10^7/ml
	T^5 62.5 µg	2694	2×10^4	5.4×10^7/ml
	T^4 125 µg	446	1×10^4	4.5×10^6/ml
	T^3 250 µg	9	4×10^3	3.6×10^4/ml
	T^2 375 µg	0	4×10^3	-
	T^1 500 µg	0	4×10^3	-

TABLE 124 (continued)

Conclusion:-	Doses for full tests	Dilution factor
	T7 3.9 µg	5×10^4
	T6 7.8 µg	5×10^4
	T5 15.63 µg	5×10^4
	T4 31.25 µg	5×10^4
	T3 62.5 µg	5×10^4
	T2 125 µg	5×10^3
	T1 250 µg	5×10^3

TABLE 125

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with diphenylamine

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Diphenylamine
Incubation time:	30 min
Activation:	-
Liver prep. date:	-
Date plated:	23 March 1979
Date counted:	4 April 1979
Plates (batch):	P80341

TABLE 125 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Diphenylamine

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	24,036	100	0	0	0.8 (2)	0	0.8 (2)	0.4 (1)	1.6 (4)	0	3.7 (9)
EMS	Dose 8 +ve control 20 mg 80.5mM	23,488	98	8.5 (20)	8.1 (19)	6.0 (14)	1.2 (3)	43.0 (101)	22.1 (52)	44.3 (104)	16.6 (39)	133.3 (313)
Diphenyl-amine	Dose 7 7.8 µg <0.1mM	23,806	99	0.4 (1)	0.4 (1)	1.7 (4)	0	0.8 (2)	1.7 (4)	1.2 (3)	0.8 (2)	6.3 (15)
	Dose 6 15.6 µg <0.1mM	23,504	98	0.8 (2)	0.4 (1)	2.1 (5)	0.4 (1)	2.5 (6)	1.3 (3)	1.3 (3)	1.3 (3)	8.9 (21)
	Dose 5 31.2 µg 0.1mM	18,780	78	0.5 (1)	0	1.1 (2)	0.5 (1)	1.6 (3)	1.1 (2)	2.7 (5)	0.5 (1)	7.4 (14)
	Dose 4 62.5 µg 0.2mM	17,832	74	1.1 (2)	1.1 (2)	1.6 (3)	1.6 (3)	1.6 (3)	1.1 (2)	2.2 (4)	2.2 (4)	10.7 (19)
	Dose 3 128 µg 0.4mM	17,566	73	1.1 (2)	0	2.3 (4)	0.5 (1)	3.4 (6)	0.5 (1)	2.3 (4)	1.1 (2)	10.2 (18)
	Dose 2 250 µg 0.7mM	8,425	35	1.1 (1)	1.1 (1)	5.9 (5)	0	5.9 (5)	2.4 (2)	8.3 (7)	2.4 (2)	24.9 (21)
	Dose 1 500 µg 1.5mM	2	<1	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate

TABLE 126

S. cerevisiae D5 Recombinogenic Activity with Diphenylamine, with Metabolic activation Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Christopher Corden
Substance:	Diphenylamine
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	29 August 1979
Date plated:	31 August 1979
Date counted:	11 September 1979
Plates (Batch):	M47142

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
Diphenylamine	3.9 µg	5×10^4
Diphenylamine	7.8 µg	5×10^4
Diphenylamine	15.6 µg	5×10^4
Diphenylamine	31.3 µg	5×10^4
Diphenylamine	62.5 µg	5×10^4
Diphenylamine	125 µg	5×10^3
Diphenylamine	250 µg	3×10^3

TABLE 126 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with Diphenylamine with metabolic activation
Modified Incubation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	7943 / 4.0×10^7	100	1.2 (1)	1.2 (1)	1.2 (1)	1.2 (1)	2.5 (2)	2.5 (2)	0	2.5 (2)	10.1 (8)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	6496 / 6.5×10^7	163	1.5 (1)	1.5 (1)	0	0	1.5 (1)	1.5 (1)	0	3.1 (2)	6.2 (4)
Diphenyl-amine	Dose 7 3.9 µg	7567 / 3.8×10^7	95	0	1.3 (1)	1.3 (1)	0	1.3 (1)	2.6 (2)	0	1.3 (1)	6.6 (5)
	Dose 6 7.8 µg	7846 / 3.9×10^7	98	0	0	2.5 (2)	0	2.5 (2)	1.3 (1)	0	0	6.4 (5)
	Dose 5 15.6 µg	8452 / 4.2×10^7	105	0	0	0	2.4 (2)	1.2 (1)	1.2 (1)	1.2 (1)	0	5.9 (5)
	Dose 4 31.3 µg	8465 / 4.2×10^7	105	1.2 (1)	0	0	1.2 (1)	2.4 (2)	0	0	1.2 (1)	4.7 (4)
	Dose 3 62.5 µg	8446 / 4.2×10^6	11	0	1.2 (1)	1.2 (1)	1.2 (1)	1.2 (1)	0	1.2 (1)	1.2 (1)	5.9 (5)
	Dose 2 125 µg	17749 / 8.9×10^6	13	0	0.6 (1)	0.6 (1)	0.6 (1)	1.7 (3)	1.1 (2)	0	0.6 (1)	4.5 (8)
	Dose 1 250 µg	12 / 3.6×10^3	<1	0	0	0	0	0	0	0	0	0

Figures in parentheses are actual number of aberrants counted

TABLE 127

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: 1,3-Dinitrobenzene
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
1,3-Dinitrobenzene	10.0 mg pptn	with S-9	22 n	23 n	24 n	21 n	20 n	19 n
		without S-9	23 n	23 n	22 n	20 n	22 n	22 n

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
pptn = precipitation
n = non specific killing

TABLE 128

Toxicity Test in Strain TA 98

Substance: 1,3-Dinitrobenzene

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R46549

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 11 December 1978

Blue = without S-9

Date counted: 13 December 1978

Culture batch: A

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	23	22
1,3-Dinitrobenzene	10.0 µg	33	45
	33.3 µg	49	71
	100.0 µg	79	292
	333.3 µg	907	1123
	1.0 mg	VTL	VTL
	3.3 mg pptn	NL	NL
	10.0 mg pptn	NL	NL

pptn = precipitation
VTL = very thin lawn
NL = no lawn i.e. complete killing

TABLE 129

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: 1,3-Dinitrobenzene

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 20 December 1978
Date counted: 22 December 1978

Activation: Aroclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): P13844
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	19 8 8	16 21 15	11 15 26	17 16 25
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	48 26 20	12 14 13	232 347 195	306 366 290
1,3-Dinitrobenzene	1.0 µg	8 6 8	5 8 7	20 12 18	23 13 12
	3.3 µg	11 10 10	8 7 8	18 17 16	12 22 25
	10.0 µg	10 7 9	3 6 6	26 21 11	24 31 29
	33.3 µg	8 8 10	9 9 4	21 26 23	34 33 55
	100.0 µg	9 7 12	6 5 11	39 41 31	73 65 89
	333.3 µg	10 19 6	16STL 13STL 12STL	802 472 472	595 548 624
	1.0 mg	NL NL NL	NL NL NL	VTL VTL VTL	VTL VTL NL

STL = slightly thin lawn: VTL = very thin lawn:
NL = no lawn i.e. complete killing

TABLE 130

Re-test of Salmonella Plate Test in Strain TA 98

Substance: 1,3-Dinitrobenzene

Project no: 410110
Activation: Aroclor-induced Fischer rat
Contractor: US Army
Liver preparation date: 11 December 1978
Operator(s): Colin Riach
Batch no. (plates): P79240
Anne Gilroy
Numbering colour(s): Red = with S-9
Date plated: 8 January 1979
Blue = without S-9
Date counted: 10 January 1979
Culture batch: C

Substance	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	22 27 21	21 18 21
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	110 180 137	326 318 424
1,3-Dinitrobenzene	31.25 µg	34 40 36	57 50 42
	62.5 µg	67 55 67	89 73 81
	125.0 µg	contam 181 257	250 365 281
	250.0 µg	433 379 386	531 502 609
	500.0 µg	902 780 1186	972 1090 1542

contam = contamination

TABLE 131

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: 1,3-Dinitrobenzene

Project no: 410110

Activation: Aroclor-induced Fischer Rat

Contractor: U.S. Army

Liver preparation date: 20 September 1979

Operator(s): Colin Riach, Chris Corden, Jennifer Harvey

Batch no. (plates): P9040

Numbering colour(s): Red with S-9
Blue without S-9

Date plated: 25 September 1979

Date counted: 27 September 1979

Culture batch: D

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	7 19 16	4 13 7	15 14 28	9 9 16	74 86 85	81 77 76
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	29 30 14	1422 1122 1503	152 175 145	296 341 314	231 289 296	241 210 234
1,3-Dinitrobenzene	1.0 µg	1 9 7	12 13 17	17 18 31	12 24 15	87 102 99	74 60 80
	3.3 µg	9 6 8	14 6 5	40 30 37	26 19 30	100 99 84	96 86 77
	10.0 µg	15 9 13	13 3 8	25 18 21	18 28 53	113 88 101	76 88 84
	33.3 µg	15 20 7	13 8 13	28 40 38	85 153 99	110 66 88	219 165 169
	100.0 µg	16 12 7	28 33 48	57 66 75	593 663 608	138 117 110	530 561 489
	333.3 mg	19 20 17	12 12 13	339 271 324	12 9 16	221 247 204	97 102 96
	1.0 mg	38 66 76	VTL VTL VTL	1909 1865 VTL	VTL VTL VTL	1033 912 976	VTL VTL VTL

VTL = very thin lawn

TABLE 132

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: 1, 3-Dinitrobenzene

Activation: Aroclor-induced Fischer rat

Date plated: 24 January 1979

Liver preparation date: 16 January 1979

Date counted: 30 January 1979

Batch no. (plates): R 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	123	116	132	117	109
1,3-Dinitro-benzene	1 mg (2.9 mM)	112	105	109	110	92
	55.1 mg (163.8 mM)	120	126	119	161	109
	110.2 mg (327.7 mM)	124	88	130	100	99

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	122	124	128	156	114
1,3-Dinitro-benzene	1 mg (2.9 mM)	122	127	138	180	104
	55.1 mg (163.8 mM)	128	144	60	118	123
	110.2 mg (327.7 mM)	126	138	101	146	108

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 133

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with 1,3-Dinitrobenzene

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	1,3-Dinitrobenzene
Incubation time:	2 h
Activation:	-
Liver prep. date:	-
Date plated:	17 April 1979
Date counted:	27 April 1979
Plates (batch):	M11218/M90304/P43142

TABLE 133 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with 1,3-Dinitrobenzene

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	19,838	100	0.5 (1)	0	3.5 (7)	3.5 (7)	2.5 (5)	1.5 (3)	4.0 (8)	0.5 (1)	15.5 (31)
EMS	Dose 8 +ve control 20 mg 80.5mM	21,864	110	38.9 (85)	33.0 (71)	12.3 (27)	3.2 (7)	128.0 (275)	25.1 (55)	86.9 (190)	71.4 (156)	324.7 (710)
1,3-Dinitro-benzene	Dose 7 2 mg 6mM	21,490	108	1.3 (3)	0.9 (2)	8.3 (18)	3.7 (8)	2.8 (6)	0.9 (2)	4.2 (9)	2.2 (5)	22.3 (48)
	Dose 6 4 mg 12mM	21,382	108	0.5 (1)	0.5 (1)	0	0.5 (1)	1.4 (3)	1.8 (4)	4.7 (10)	1.0 (2)	9.4 (20)
	Dose 5 8 mg 24mM	23,846	120	0.4 (1)	0	0.8 (2)	0.4 (1)	1.2 (3)	1.2 (3)	2.5 (6)	0.4 (1)	6.2 (15)
	Dose 4 16 mg 48mM	24,272	125	0.8 (2)	0	0	0.4 (1)	0.8 (2)	0	0.8 (2)	0.8 (2)	2.8 (7)
	Dose 3 32 mg 95mM	22,928	116	0	0	0	0.8 (2)	0	0	0.4 (1)	0	1.2 (3)
	Dose 2 64 mg 190mM	22,774	115	0.4 (1)	0.4 (1)	0.4 (1)	1.7 (4)	2.2 (5)	2.6 (6)	3.5 (8)	0.8 (2)	11.4 (26)
	Dose 1 134.3 mg 399mM	21,432	108	0	0.5 (1)	0.9 (2)	1.3 (3)	2.7 (6)	1.3 (3)	3.7 (8)	0.5 (1)	10.7 (23)

Figures in parenthesis are actual number of aberrants counted DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphoxide
pptn = precipitation

TABLE 134

Saccharomyces cerevisiae D5 Recombinogenic Activity
with metabolic activation with 1,3-Dinitrobenzene

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	1,3,5-Dinitrobenzene
Incubation time:	18 h (modified method)
Activation:	Aroclor-induced Fischer rat
Liver prep. date:	18 June 1979
Date plated:	6 July 1979
Date counted:	17 July 1979
Plates (batch):	R60541

TABLE 134 (continued)

Estimation of cell numbers (All counts are approximate and are visible not viable count)

Visible count before incubation of stock culture (i.e. using counting chamber) = 1×10^7 cells/ml

Viable count after 18 h incubation

DMSO 100 µl		6.25×10^7/ml
Cyclophosphamide	40.45 mg	8.1×10^7/ml
1,3-DNB -	1 mg	1.0×10^7/ml
	2 mg	1.1×10^7/ml
	4 mg	1.2×10^7/ml
	8 mg	1.5×10^7/ml
	16 mg	1.4×10^7/ml
	32 mg	1.5×10^7/ml
	64 mg	1.2×10^7/ml

Dilution factor =

DMSO	2×10^4
Cyclophosphamide	2.6×10^4
1,3-DNB - 1 mg	3.3×10^3
2 mg	3.3×10^3
4 mg	3×10^3
8 mg	4×10^3
16 mg	4×10^3
32 mg	4×10^3
64 mg	3.3×10^3

TABLE 134 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with metabolic activation with 1,3-Dinitrobenzene

Substance	Dose	No. of Survivors: viable count after 18h incubation	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	12,951: $3.0x10^7$	100	1.5 (2)	2.3 (3)	1.5 (2)	0	3.1 (4)	3.1 (4)	3.8 (5)	3.8 (5)	15.4 (20)
Cyclophosphamide	Dose 8 +ve control 10.45mg 72 mM	21,983: $5.7x10^7$	190	2.7 (6)	5.4 (12)	1.8 (4)	0.5 (1)	5.0 (11)	2.3 (5)	2.7 (6)	8.2 (18)	20.1 (45)
1,3-Dinitrobenzene	Dose 7 1 mg 3 mM	24,943: $8.2x10^6$	27	0	0.8 (2)	2.8 (7)	0.8 (2)	1.2 (3)	1.2 (3)	1.2 (3)	0.8 (2)	8.0 (20)
	Dose 6 2 mg 6 mM	18,151: $6.0x10^6$	20	2.8 (5)	1.1 (2)	2.2 (4)	0.6 (1)	1.1 (2)	0.6 (1)	0.6 (1)	3.9 (7)	8.8 (16)
	Dose 5 4 mg 12 mM	14,970: $4.5x10^6$	15	2.0 (3)	2.0 (3)	1.3 (2)	0	3.3 (5)	2.7 (4)	2.0 (3)	4.0 (6)	13.4 (20)
	Dose 4 8 mg 24 mM	12,816: $5.1x10^6$	17 pptn	3.1 (4)	1.5 (2)	2.3 (3)	2.3 (3)	3.9 (5)	0.9 (1)	2.3 (3)	4.7 (6)	16.4 (21)
	Dose 3 16 mg 48 mM	13,406: $5.4x10^6$	18 pptn	2.2 (3)	3.0 (4)	1.5 (2)	0	4.4 (6)	6.0 (8)	0.7 (1)	5.2 (7)	17.9 (24)
	Dose 2 32 mg 95 mM	12,836: $5.1x10^6$	17 pptn	3.1 (4)	4.7 (6)	2.3 (3)	3.1 (4)	6.2 (8)	2.3 (3)	1.6 (2)	7.8 (10)	23.3 (30)
	Dose 64 mg 140 mM	17,185: $5.7x10^6$	19 pptn	2.3 (4)	1.7 (3)	1.2 (2)	1.7 (3)	4.0 (7)	2.3 (4)	2.3 (4)	4.0 (7)	15.7 (27)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide

TABLE 135

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: 1,3,5-Trinitrobenzene
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per Plate	Activation	pol A^+ (100 µl)			pol A^- (100 µl)		
1,3,5-Trinitrobenzene	10.0 mg pptn	with S-9	30 n	29 n	29 n	29 n	31 n	30 n
		without S-9	30 n	34 n	30 n	32 n	33 n	30 n

Measurements in mm Diameter of hole = 15 mm

controls as for Table 2
pptn = precipitation
n = non specific killing

TABLE 136

Toxicity Test in Strain TA 98

Substance: 1,3,5-Trinitrobenzene

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 11 December 1978
Date examined: 13 December 1978

Activation: Arcclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): R46549
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: A

Toxicity Test	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	23	22
Tube I.D.	Quantity		
G	10.0 µg	37	115
F	33.3 µg	144	592STL
E	100.0 µg	contam STL	VTL
D	333.3 µg	VTL	NL
C	1.0 mg	NL	NL
B	3.3 mg pptn	NL	NL
A	10.0 mg pptn	NL	NL

STL = slightly thin lawn
VTL = very thin lawn
NL = complete killing
pptn = precipitation
contam = contamination

TABLE 137

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: 1,3,5-Trinitrobenzene

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 8 January 1979
Date counted: 10 January 1979

Activation: Aroclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): P88340
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	11 9 10	8 11 11	22 27 21	21 18 21
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	14 19 24	129 206 182	110 180 137	326 318 424
1,3,5-Trinitrobenzene	0.1 µg	10 8 17	11 7 8	28 29 19	27 25 20
	0.3 µg	17 9 11	11 14 12	23 23 23	21 33 41
	1.0 µg	13 11 10	10 18 15	23 28 29	47 50 41
	3.3 µg	15 18 12	9 13 9	32 27 28	60 34 58
	10.0 µg	9 8 13	24 8 5	38 38 33	95 110 94
	33.3 µg	22 13 12	39STL 28STL 37STL	87 101 86	462 432 369
	100.0 µg	33 35 30	VTL VTL VTL	644 781 714	TL* 291TL 387TL

STL = slightly thin lawn
TL = thin lawn
VTL = very thin lawn
* plate unevenly poured

TABLE 138

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Substance: 1,3,5-Trinitrobenzene

Project no: 410110 Activation: Aroclor-induced Fischer Rat

Contractor: U.S. Army Liver preparation date: 20 September 1979

Operator(s): Colin Riach, Chris Batch no. (plates): P9040

Corden, Jennifer Harvey Numbering colour(s): Red with S-9

Date plated: 25 September 1979 Blue without S-9

Date counted: 27 September 1979 Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	7 19 16	4 13 7	15 14 28	9 9 16	74 86 85	81 77 76
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	29 30 14	1422 1122 1503	1152 175 145	296 341 314	231 289 296	241 101 234
1,3,5-Trinitrobenzene	0.1 µg	15 15 6	16 15 15	27 30 30	18 14 28	84 102 65	100 101 100
	0.3 µg	5 14 8	15 26 12	25 21 27	39 25 21	89 76 93	89 124 92
	1.0 µg	9 16 9	8 13 16	31 25 27	17 25 18	103 89 89	114 91 115
	3.3 µg	7 5 7	20 17 16	27 27 24	31 50 78	102 98 126	135 165 160
	10.0 µg	15 13 16	60 39 28	36 57 51	162 151 TL	 contam	281 277 297
	33.3 µg	12 30 17	TL TL TL	142 127 159	TL TL TL	277 280 272	717 819 497
	100.0 µg	44 43 48	VTL VTL VTL	127 141 173	VTL VTL VTL	496 384 417	TL TL TL

TL = thin lawn

VTL = very thin lawn

contam = contamination

TABLE 139

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: 1,3,5-Trinitrobenzene

Activation: Aroclor-induced Fischer rat

Date plated: 24 January 1979

Liver preparation date: 16 January 1979

Date counted: 30 January 1979

Batch no. (plates): M 93940

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	120	134	155	157	155
1,3,5-Trinitro-benzene	1 mg (2.3 mM)	124	112	126	97	67
	66.7 mg (156.4 mM)	125	104	69	65	55
	133.4 (312.9 mM)	142	140	76	61	67

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	155	155	154	144	181
1,3,5-Trinitro-benzene	1 mg (2.3 mM)	125	106	116	97	88
	66.7 mg (156.4 mM)	160	118	98	70	73
	133.4 mg (312.9 mM)	129	113	201	103	102

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 140

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Substance: 1,3,5-Trinitrobenzene

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 18 June 1979

Operator(s): Rowan Hastwell

Batch no. (plates): BO1441

Jennifer Harvey

Numbering System X

Date plated: 20 July 1979

Date counted: 26 July 1979

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2410	2×10^4	4.8×10^7/ml
1,3,5-Trinitrobenzene	X^7 125 µg	102	4×10^3	3.1×10^5/ml
	X^6 250 µg	24	4×10^3	9.6×10^4/ml
	X^5 500 µg	20	4×10^3	8.0×10^4/ml
	X^4 1 mg	14	1×10^3	1.4×10^4/ml
	X^3 2 mg	2	1×10^4	2×10^4/ml
	X^2 4 mg pptn	0	1×10^4	-
	X^1 8 mg pptn	1	1×10^2	1×10^2/ml

pptn = Precipitation

TABLE 140 (continued)

Conclusion:-	Doses for full test	Dilution factor
	X7 15.63 µg	1×10^4
	X6 31.25 µg	1×10^3
	X5 62.5 µg	1×10^2
	X4 125 ug	1×10^2
	X3 250 ug	4 x 10
	X2 500 µg	4 x 10
	X1 1 mg	X 7

TABLE 141

Saccharomyces cerevisiae D5 Recombinogenic
Activity without activation, with 1,3,5-Trinitrobenzene

Project No:	410110
Contractor:	U.S. Army
Operators:	Colin Riach Jennifer Harvey
Substance:	1,3,5-Trinitrobenzene
Incubation time:	2 h
Activation:	-
Liver prep. date:	-
Date plated:	20 April 1979
Date counted:	2 May 1979
Plates (batch):	M93003/P43142

TABLE 141 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with 1,3,5-Trinitrobenzene

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	10,992	100	1.8 (2)	0.9 (1)	1.8 (2)	0.9 (1)	0.9 (1)	1.8 (2)	3.6 (4)	2.7 (3)	11.1 (13)
EMS	Dose 8 +ve control 20 mg 80.5mM	10,692	97	24.3 (26)	11.2 (12)	5.6 (6)	2.8 (3)	38.3 (41)	22.4 (24)	131.9 (141)	35.5 (38)	236.5 (153)
1,3,5-Trinitrobenzene	Dose 7 2 mg 5mM	11,663	106	0.9 (1)	1.7 (2)	1.7 (2)	0.9 (1)	0	0.9 (1)	3.4 (4)	2.6 (3)	9.5 (11)
	Dose 6 4 mg 9mM	11,683 pptn	106	0.9 (1)	0.9 (1)	0	1.7 (2)	1.7 (2)	0	5.1 (6)	1.8 (2)	9.2 (12)
	Dose 5 8 mg 19mM	13,106 pptn	119	1.5 (2)	0.8 (1)	0.8 (1)	0.8 (1)	1.5 (2)	2.2 (3)	4.6 (6)	2.3 (3)	12.2 (16)
	Dose 4 16 mg 38mM	9,919 pptn	90	2.0 (2)	1.0 (1)	2.0 (2)	1.0 (1)	9.1 (9)	2.0 (2)	8.1 (8)	3.0 (3)	25.2 (25)
	Dose 3 32 mg 75mM	9,154 pptn	83	1.1 (1)	4.4 (4)	6.6 (6)	1.1 (1)	6.6 (6)	4.4 (4)	10.9 (10)	5.5 (5)	34.9 (32)
	Dose 2 64 mg 150mM	11,965 pptn	109	0.8 (1)	3.3 (4)	0.8 (1)	1.7 (2)	0.8 (1)	0	5.0 (6)	4.1 (5)	12.5 (15)
	Dose 1 119.1 mg 279mM	10,616 pptn	97	0	1.9 (2)	0.9 (1)	0	1.9 (2)	0	5.7 (6)	1.9 (2)	10.3 (11)

Figures in parentheses are actual number of aberrants counted

TABLE 142

Saccharomyces cerevisiae D5 Recombinogenic Activity
with metabolic activation with 1,3,5-Trinitrobenzene

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	1,3,5-Trinitrobenzene
Incubation time:	18 h (modified method)
Activation:	Aroclor-induced Fischer rat
Liver prep. date:	18 June 1979
Date plated:	13 July 1979
Date counted:	23 July 1979
Plates (batch):	B01441

TABLE 142 (continued)

Estimation of cell numbers: (All counts are approximate and are visible not viable count)

Visible count before incubation of stock culture (i.e. using counting chamber) = 1×10^7 cells/ml.

Visible count after 18 h incubation

DMSO 100 µl 6.7×10^7 cells/ml
Cyclophosphamide 39.8 mg 1.0×10^8 cells/ml

Dilution of incubation mix for plating:

1. DMSO

A 6.7×10^7/ml
B 7×10^5/ ml 0.1 ml A + 9.9 ml phosphate buffer
C 7×10^3/ ml 0.1 ml B + 9.9 ml phosphate buffer
C 3×10^3/ml 6 ml C + 8 ml phosphate buffer

2. Cyclophosphamide

A 1×10^3/ml
B 1×10^6/ml 0.1 ml A + 9.9 ml phosphate buffer
C 1×10^4/ml 0.1 ml B + 9.9 ml phosphate buffer
D 3×10^3/ml 4.5 ml C + 10.5 ml phosphate buffer

TABLE 142 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with metabolic activation, with 1,3,5-Trinitrobenzene

Substance	Dose	No. survivors ± viable count after 18h incubation	survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 +ve control 200 µl	24089: 5.5×10^7/ml	100	0	0.4 (1)	0.8 (2)	0.4 (1)	0.8 (2)	0.8 (2)	2.1 (5)	0.4 (1)	5.4 (13)
Cyclophosphamide	Dose 8 +ve control 39.8 mg 71 mM	19520: 6.4×10^7/ml	116	5.1 *(10)*	6.1 *(12)*	2.6 *(5)*	0.5 *(1)*	7.2 *(14)*	2.0 *(4)*	2.6 *(5)*	11.3 (22)	26.1 (51)
1,3,5-Trinitrobenzene	Dose 7 1 mg 4 mM	*										
	Dose 6 2 mg 7 mM	*										
	Dose 5 4 mg 15 mM	*										
	Dose 4 8 mg 29 mM	*										
	Dose 3 16 mg 59 mM	*										
	Dose 2 32 mg 117 mM	*										
	Dose 1 64 mg 235 mM	*										

Figures in parentheses are actual number of aberrants counted

* Survival very low i.e. less than 1%, therefore aberrants and colonies not counted

DMSO = Dimethylsulphoxide

TABLE 143

S. cerevisiae D5 Recombinogenic Activity with
1,3,5-Trinitrobenzene, with Metabolic activation
Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	1,3,5-Trinitrobenzene
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	15 August 1979
Date plated:	29 August 1979
Date counted:	17 September 1979
Plates (Batch):	B01441

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
1,3,5-Trinitrobenzene	15.6 µg	1×10^4
1,3,5-Trinitrobenzene	31.3 µg	5×10^3
1,3,5-Trinitrobenzene	62.5 µg	1×10^3
1,3,5-Trinitrobenzene	125.0 µg	1×10^2
1,3,5-Trinitrobenzene	250.0 µg	4 x 10
1,3,5-Trinitrobenzene	500.0 µg	4 x 10
1,3,5-Trinitrobenzene	1.0 mg	x7

TABLE 143 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with 1,3,5 -Trinitrobenzene, with metabolic activation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	9539 / 4.8×10^7	100	0	3.1 (3)	1.0 (1)	0	1.0 (1)	0	1.0 (1)	3.1 (3)	6.3 (6)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	8534 / 8.5×10^7	177	1.2 (1)	0	1.2 (1)	0	0	0	0	1.2 (1)	2.3 (2)
1,3,5-Trinitro-Benzene	Dose 7 15.63 µg	29,797 / 3.0×10^7	62.5	0	0	0.6 (2)	0	0.6 (2)	0	0	0	1.3 (4)
	Dose 6 31.25 µg	44119 / 2.2×10^6	4.6	0	0.5 (2)	0	0	0	0	0.2 (1)	0.9 (2)	0.7 (3)
	Dose 5 62.5 µg	*										
	Dose 4 125.0 µg	*										
	Dose 3 250.0 µg	36576 / 1.5×10^5	<1	0	0.3 (1)	0.3 (1)	0	0	0	0	0.3 (1)	0.5 (2)
	Dose 2 500.0 µg	4317 / 1.7×10^4	<1	0	2.0 (1)	0	2.0 (1)	0	0	0	2.3 (1)	4.6 (2)
	Dose 1 1.0 mg	15996 / 1.1×10^4	<1	0	0	2.5 (4)	0.6 (1)	0.6 (1)	0	0	0	3.7 (6)

Figures in parentheses are actual number of aberrants counted

* Doses 4 and 5:- Plates on these doses contained over 600 colonies each, making accurate scoring of aberrants impossible

TABLE 144

E. coli Toxicity Test

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 26 January 1979
Date examined: 28 January 1979

Substance: Zinc chloride
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P30141
Numbering colour(s): Red = with S-9
Blue = without S-9

Toxicity	Quantity per plate	Activation	pol A^+ (100 µl)			pol A^- (200 µl)		
Zinc chloride	10.0 mg	with S-9	21 s	20 s	20 s	21 s	20 s	22 s
		without S-9	17 s	21 s	23 s	23 s	22 s	22 s

Measurements in mm Diameter of hole = 15 mm

controls as for Table 21
s = specific killing

TABLE 145

Toxicity Test in Strain TA 98

Substance: Zinc chloride

Project no: 410110

Activation: Aroclor-induced Fischer rat

Contractor: US Army

Liver preparation date: 11 December 1978

Operator(s): Colin Riach

Batch no. (plates): R46549

Anne Gilroy

Numbering colour(s): Red = with S-9

Date plated: 11 December 1978

Blue = without S-9

Date examined: 13 December 1978

Culture batch: A

Toxicity Test	Quantity per Plate	TA 98	
		with S-9	without S-9
Dimethylsulphoxide	100 µl	23	22
Tube I.D.	Quantity		
G	10.0 µg	39	29
F	33.3 µg	40	26
E	100.0 µg	38	27
D	333.3 µg	33	18
C	1.0 mg	31	16TL
B	3.3 mg pptn	33TL	27TL
A	10.0 mg pptn	VTL	TL

TL = thin lawn
VTL = very thin lawn
pptn = precipitation

TABLE 146

Salmonella Plate Test in Strains TA 1535 and TA 98

Substance: Zinc chloride

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach
Anne Gilroy
Date plated: 8 January 1979
Date counted: 10 January 1979

Activation: Arcclor-induced Fischer rat
Liver preparation date: 11 December 1978
Batch no. (plates): P88340
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: B

Substance	Quantity per Plate	TA 1535		TA 98	
		with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	11 9 10	8 11 11	22 27 21	21 18 21
2-Aminoanthracene Sodium azide 2-Nitrofluorene	0.5 µg 0.5 µg 2.0 µg	14 19 24	129 206 182	110 180 137	326 318 424
Zinc chloride	3.3 µg	9 11 12	8 11 12	22 17 27	26 36 26
	10.0 µg	15 11 14	12 12 15	23 22 30	19 23 22
	33.3 µg	22 7 10	8 8 11	20 29 22	16 18 34
	100.0 µg	10 14 13	8 9 6	14 19 19	16 24 29
	333.3 µg	11 14 11	10 13 17	27 22 27	26 22 19
	1.0 mg	13 13 8	10 14 17	22 31 22	29TL 21TL 25TL
	3.3 mg	13TL 14TL 12TL	9TL 13TL 15TL	23TL 25TL 26TL	VTL VTL VTL

TL = thin lawn
VTL = very thin lawn

TABLE 147

Salmonella Plate Test in Strains TA 1537, TA 1538 and TA 100

Project no: 410110
Contractor: US Army
Operator(s): Colin Riach

Date plated: 24 January 1979
Date counted: 26 January 1979

Substance: Zinc chloride
Activation: Aroclor-induced Fischer rat
Liver preparation date: 16 January 1979
Batch no. (plates): P90142
Numbering colour(s): Red = with S-9
Blue = without S-9
Culture batch: C

Substance	Quantity per Plate	TA 1537		TA 1538		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Dimethylsulphoxide	100 µl	2 6 4	11 13 8	26 contam 34	35 34 29	120 149 115	129 129 139
2-Aminoanthracene 9-Aminoacridine 2-Nitrofluorene Sodium azide	0.5 µg 50.0 µg 2.0 µg 0.5 µg	17 19 12	1099 855 991	268 305 242	534 524 621	264 242 258	260 281 300
Zinc chloride	3.3 µg	3 11 5	7 13 4	26 28 27	contam 26 23	138 108 114	130 130 115
	10.0 µg	11 11 7	contam 8 12	36 28 42	24 35 26	123 128 146	122 146 136
	33.3 µg	7 9 13	13 11 14	23 27 34	30 33 31	131 107 103	142 100 152
	100.0 µg	13 15 7	13 11 9	36 34 26	42 20 27	118 147 138	126 125 141
	333.3 µg	15 11 9	7 12 4	40 30 37	30 38 22	149 137 120	169 163 177
	1.0 mg	9 9 12	7TL 9TL 11TL	38 33 25	25STL 33STL 34STL	138 142 152	147STL 130STL 105STL
	3.3 mg	8TL 8TL 11TL	14TL 10TL 6TL	29STL 27STL 36STL	26TL 36TL 36TL	134TL 150TL 139TL	114TL 135TL 105TL

contam = contamination
STL = slightly thin lawn
TL = thin lawn

TABLE 148

Saccharomyces cerevisiae D5 Toxicity Test

Mean number of colonies from five plates
at each dose and sampling time

Project no: 410110

Contractor: US Army

Operator(s): Rowan Hastwell
Anne Gilroy

Substance: Zinc chloride

Activation: Aroclor-induced Fischer rat

Date plated: 26 January 1979

Liver preparation date: 16 January 1979

Date counted: 1 February 1979

Batch no. (plates): M 27540

1. With activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	151	165	159	168	153
Zinc chloride	1 mg (3.6 mM)	167	144	164	148	149
	44.8 mg (164.3 mM)	142	140	117	127	94
	89.7 mg (329.0 mM)	147	140	149	118	88

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

2. Without activation

Incubation Time		30 min	60 min	90 min	120 min	150 min
Substance	Quantity					
Dimethylsulphoxide	200 µl	181	169	145	151	110
Zinc chloride	1 mg (3.6 mM)	174	163	188	168	163
	44.8 mg (164.3 mM)	107	128	94	74	89
	89.7 mg (329.0 mM)	126	148	144	135	159

Conclusion: Dose range: Saturation, 64 mg, 32 mg, 16 mg, 8 mg, 4 mg, 2 mg
Incubation time: 2 h

TABLE 149

Toxicity Test in S. cerevisiae D5 - 18 h incubation with Metabolic Activation

Project no: 410110
Contractor: US Army
Operator(s): Rowan Hastwell
Jennifer Harvey
Date plated: 20 July 1979
Date counted: 26 July 1979

Substance: Zinc chloride
Activation: Aroclor-induced Fischer rat
Liver preparation date: 18 June 1979
Batch no. (plates): BO1441
Numbering System: Z

Substance	Dose	Counts from 10 plates	Dilution factor used	Viable count after 18 h incubation
Dimethylsulphoxide	100 µl	2410	2×10^4	4.8×10^7/ml
Zinc chloride	$Z3_{1.5}$ mg	315	1×10^4	3.1×10^6/ml
	$Z2_{15}$ mg pptn	8	1×10^4	8×10^4/ml
	$Z1_{30}$ mg pptn	17	1×10^4	1.7×10^5/ml

pptn = precipitation

TABLE 149 (continued)

Conclusion:-	Doses for full test	Dilution factor
	Z7 500 µg	5×10^3
	Z6 1 mg	3×10^3
	Z5 2 mg	3×10^3
	Z4 4 mg	1×10^3
	Z3 8 mg	5×10^2
	Z2 16 mg	1×10^2
	Z1 32 mg	1×10^2

TABLE 150

Saccharomyces cerevisiae D5 Recombinogenic Activity without activation, with zinc chloride

Project No.:	410110
Contractor:	US Army
Operators:	Rowan Hastwell Jennifer Harvey
Substance:	Zinc chloride
Incubation time:	2 h
Activation:	-
Liver prep. date:	-
Date plated:	6 April 1979
Date counted:	18 April 1979
Plates (batch):	R40341

TABLE 150 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
without activation, with Zinc chloride

		No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO	Dose 9 -ve control 200 µl	16,239	100	0.6 (1)	1.2 (2)	0.6 (1)	0	0	1.2 (2)	2.5 (4)	1.8 (3)	6.1 (10)
EMS	Dose 8 +ve control 20 mg 80.5mM	16,806	103	26.8 (45)	36.9 (62)	14.3 (24)	7.1 (12)	79.1 (133)	73.2 (123)	108.9 (183)	63.7 (107)	346.3 (582)
Zinc chloride	Dose 7 2 mg 7mM	12,839 pptn	79	0.8 (1)	0.8 (1)	0	0.8 (1)	0.8 (1)	0.8 (1)	3.9 (5)	0.8 (2)	7.1 (10)
	Dose 6 4 mg 15mM	14,457 pptn	89	1.4 (2)	1.4 (2)	1.4 (2)	1.4 (2)	1.4 (2)	0.7 (1)	3.5 (5)	2.8 (4)	11.2 (16)
	Dose 5 8 mg 29mM	5,108 pptn	31	0	0	1.9 (1)	0	1.9 (1)	5.9 (3)	11.7 (6)	0	21.4 (11)
	Dose 4 16 mg 59mM	7,970 pptn	49	3.8 (3)	2.5 (2)	0	0	3.8 (3)	6.3 (5)	7.5 (6)	6.3 (5)	23.9 (19)
	Dose 3 32 mg 117 mM	6,957 pptn	43	0	1.4 (1)	2.9 (2)	1.4 (1)	1.4 (1)	7.2 (5)	8.6 (6)	1.4 (1)	22.9 (16)
	Dose 2 64 mg 325 mM	5,410 pptn	33	1.8 (1)	3.7 (2)	5.5 (3)	0	0	3.7 (2)	5.5 (3)	5.5 (3)	20.2 (11)
	87.8 mg 322 mM	8,770 pptn	54	0	1.1 (1)	1.1 (1)	1.1 (1)	0	0	1.1 (1)	1.1 (1)	4.4 (4)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide EMS = Ethyl methanesulphonate

pptn = precipitation

TABLE 151

S. cerevisiae D5 Recombinogenic Activity with with Zinc chloride, with Metabolic activation Modified Incubation

Project No.:	410110
Contractor:	US Army
Operators:	Christopher Corden Jennifer Harvey
Substance:	Zinc chloride
Incubation time:	Modified 18 h
Activation:	Aroclor-induced Fischer rat
Liver preparation date:	29 August 1979
Date plated:	19 September 1979
Date counted:	28 September 1979
Plates (Batch):	B10841

Dilution factors used to dilute incubation tubes after 18 h incubation.

Substance	Dose	Dilution Factor
Dimethylsulphoxide	100 µl	5×10^4
Cyclophosphamide	40 mg	1×10^5
Zinc chloride	500 µg	5×10^3
Zinc chloride	1 mg	3×10^3
Zinc chloride	2 mg	3×10^3
Zinc chloride	4 mg	1×10^3
Zinc chloride	8 mg	5×10^2
Zinc chloride	16 mg	1×10^2
Zinc chloride	32 mg	1×10^2

TABLE 151 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with Zinc chloride , with metabolic activation

Substance	Dose	No. survivors / survival	% survival	No. of aberrants/10^4 survivors: Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline	Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
Dimethyl-sulphoxide	Dose 9 -ve control 100 µl	8838 / 4.4×10^7	100	0	2.3 (2)	1.1 (1)	0	2.3 (2)	0	2.3 (2)	2.3 (2)	7.9 (7)
Cyclo-phospha-mide	Dose 8 +ve control 40 mg	5624 / 5.6×10^7	127	3.6 (2)	1.8 (1)	7.1 (4)	1.8 (1)	1.8 (1)	1.8 (1)	1.8 (1)	5.3 (3)	19.6 (11)
Zinc chloride	Dose 7 500 µg	45515 / 2.2×10^7	50	0	0.4 (2)	0.2 (1)	0	0.2 (1)	0.2 (1)	0	0.4 (2)	1.1 (5)
	Dose 6 1 mg	49522 / 1.5×10^7	34	0.2 (1)	0	0.2 (1)	0	0	0	0	0.2 (1)	0.4 (2)
	Dose 5 2 mg	10067 / 3×10^6	7	0	1.0 (1)	4.0 (4)	1.0 (1)	0	2.0 (2)	1.0 (1)	1.0 (1)	8.9 (9)
	Dose 4 4 mg	271 / 2.7×10^5	<1	0	0	0	0	0	0	0	0	0
	Dose 3 8 mg	6883 / 3.4×10^5	<1	0	2.9 (2)	5.8 (4)	1.5 (1)	0	1.5 (1)	0	2.9 (2)	11.6 (8)
	Dose 2 16 mg pptn	602 / 6×10^3	<1	0	0	16.7 (1)	16.7 (1)	0	16.7 (1)	0	0	49.8 (3)
	Dose 1 32 mg pptn	151 / 1.5×10^3	<1	0	0	0	66.2	0 (1)	0	0	0	66.2 (1)

Figures in parentheses are actual number of aberrants counted
pptn = precipitation

TABLE 152

S. cerevisiae D-5 Sensitivity Check with EMS

Project No.:	410110
Contractor:	US Army
Operators:	Rowan Hastwell
	Colin Riach
Substance:	Ethyl methanesulphonate
Incubation time:	30 min
Activation:	Without activation
Liver preparation date:	-
Date plated:	5 December 1978
Date counted:	27 and 28 December 1978

TABLE 152 (continued)

Saccharomyces cerevisiae D-5 Sensitivity Check with EMS

Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
			Pink-red	Pink-red pink	Pink	Red	White-pink	White-red	Hairline		
Dose J [illegible] 600 µl EMS [illegible]	14,162	100	0	0	0	0	0	0	0	0	0
[illegible] G [illegible] EMS	14,212	100	4.2 (6)	7 (1)	0 (0)	1.4 (2)	1.4 (2)	6.3 (9)	3.5 (5)	4.9	17.5
Dose F 20 mg EMS	8,765	66.5	15.9 (14)	23.9 (21)	3.4 (3)	2.2 (2)	53.6 (47)	30.8 (27)	21.6 (19)	39.9	151.7
Dose E 40 mg EMS	11,377	86.4	17.5 (20)	12.3 (14)	5.2 (6)	5.2 (6)	24.6 (28)	29.0 (33)	24.6 (28)	29.8	113.6
Dose D 80 mg EMS	9,202	69.9	29.3 (27)	3.2 (3)	17.3 (16)	24.9 (23)	15.2 (14)	57.5 (53)	7.6 (7)	32.6	147.7
Dose C 160 mg EMS	1,583	12.0	120.0 (19)	31.5 (5)	69.4 (11)	101.0 (16)	82.1 (13)	113.7 (18)	12.6 (2)	151.6	530.6
Dose B 240 mg EMS	36	0.2	0 (0)	0 (0)	277.7 (1)	277.7 (1)	555.5 (2)	0 (0)	277.7 (1)	0	1388.8
Dose A 320 mg EMS	10	0.0	0 (0)	0 (0)	200.0 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0	2000.0

Figures in parentheses are actual number of aberrants counted

TABLE 153

Saccharomyces cerevisiae D5 Recombinogenic Activity with metabolic activation and ethyl methanesulphonate and dimethylnitrosamine

Project No.:	410110
Contractor:	US Army
Operators:	Rowan Hastwell Jennifer Harvey
Substance:	Ethyl methanesulphonate and Dimethylnitrosamine
Incubation time:	30 min
Activation:	Aroclor-induced Fischer rat
Liver prep. date:	14 March 1979
Date plated:	26 March 1979
Date counted:	6 April 1979
Plates (batch):	R40341

AD-A084 121 INVERESK RESEARCH INTERNATIONAL LTD EDINBURGH (SCOTLAND) F/G 6/5
MUTAGENICITY AND DNA REPAIR POTENTIAL OF 15 CHEMICALS.(U)
JAN 80 D B MCGREGOR DAMD17-78-C-8064

UNCLASSIFIED NL

END
DATE FILMED
6 80
DTIC

TABLE 153 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with metabolic activation, with positive controls

Substance /Dose	S-9 co-factors	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO 200 µl	-ve control	17,938	100	0	0	0	0	1.1 (2)	1.1 (2)	0	0	2.2 (4)
EMS 20 mg 80.5 mM	+ve control	16,242	91	4.9 (8)	3.1 (5)	5.5 (9)	0	46.2 (75)	12.3 (20)	35.1 (57)	8.0 (13)	107.1 (174)
EMS 20 mg 80.5 mM	1:9	12,292	69	4.1 (5)	11.4 (14)	1.6 (2)	3.3 (4)	48.8 (60)	30.9 (38)	51.3 (63)	15.5 (19)	166.0 (204)
	1:3	11,940	67	6.7 (8)	7.5 (9)	6.7 (8)	0.8 (1)	43.5 (52)	29.3 (35)	21.8 (26)	14.2 (17)	116.4 (139)
	1:1	13,791	77	0.7 (1)	2.2 (3)	8.0 (11)	0.7 (1)	32.6 (45)	28.3 (39)	27.6 (38)	2.9 (4)	100.1 (138)
DMN 20 mg 135.0 mM	1:9	14,812	83	0	0	2.7 (4)	0	0	0	0	0	2.7 (4)
	1:3	5,215	29	1.9 (1)	0	1.9 (1)	0	1.9 (1)	1.9 (1)	1.9 (1)	1.9 (1)	9.6 (5)
	1:1	1,236	7	8.1 (1)	0	0	0	8.1 (1)	0	8.1 (1)	8.1 (1)	24.3 (3)

Figures in parentheses are actual number of aberrants counted

EMS = ethyl methanesulphonate

DMN = dimethylnitrosamine

DMSO = dimethyl sulphoxide

TABLE 154

Saccharomyces cerevisiae D5 Recombinogenic Activity with activation, with Positive Controls

Project No:	410110
Contractor:	U.S. Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Ethyl methanesulphonate Hydroxylamine hydrochloride
Incubation time:	2 h
Activation:	Aroclor-induced Fischer Rat
Liver prep. date:	11 April 1979
Date plated:	11 May 1979
Date counted:	22 May 1979
Plates (batch):	R80441

TABLE 154 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity with activation, with Positive Controls

Substance /Dose	S-9 co-factors	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Dimethyl-sulphoxide 200 µl	Dose 9 -	18,423	100	0	0.5 (1)	2.7 (5)	0	2.7 (5)	1.1 (2)	4.3 (8)	0.5 (1)	11.3 (21)
	Dose 8 1:3	21,921	100	0	0	2.7 (6)	2.3 (5)	1.4 (3)	3.2 (7)	5.5 (12)	0	15.1 (3)
	Dose 7 1:9	21,986	100	0	0.5 (1)	3.2 (7)	0	1.4 (3)	3.2 (7)	4.1 (9)	0.5 (1)	12.3 (27)
Ethyl methane-sulphonate 20 mg 805 mM	Dose 6 -	20,841	113	22.1 (46)	16.8 (35)	2.9 (6)	2.9 (6)	43.7 (91)	34.1 (71)	74.9 (159)	38.9 (81)	198.6 (414)
	Dose 5 1:3	23,429	107	28.6 (67)	15.4 (36)	7.3 (17)	3.4 (8)	41.8 (98)	43.1 (101)	63.6 (149)	43.9 (103)	203.2 (476)
	Dose 4 1:9	26,862	122	27.2 (73)	11.5 (31)	3.0 (8)	7.1 (19)	33.9 (91)	37.2 (100)	67.4 (181)	38.7 (104)	187.2 (503)
Hydroxy-lamine hydro - chloride 400 µg	Dose 3 -	26,072	142	0.4 (1)	0	0.4 (1)	1.5 (4)	0.8 (2)	2.3 (6)	1.9 (5)	0.4 (1)	7.3 (19)
	Dose 2 1:3	25,974	118	0.8 (2)	0.4 (1)	0.4 (1)	1.5 (4)	0.8 (2)	1.9 (5)	2.3 (6)	1.2 (3)	8.1 (21)
	Dose 1 1:9	25,293	115	0	0.4 (1)	0.8 (2)	0.8 (2)	0.4 (1)	3.2 (8)	2.4 (6)	0.4 (1)	7.9 (20)

Figures in parentheses are actual number of aberrants counted

TABLE 155

Saccharomyces cerevisiae D5 Recombinogenic Activity
with and without metabolic activation, with Aflatoxin B_1

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Aflatoxin B_1
Incubation time:	2 h
Activation:	Aroclor-induced Fischer rat
Liver prep. date:	18 May 1979
Date plated:	15 June 1979
Date counted:	25 June 1979
Plates (batch):	B04040

TABLE 155 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with and without metabolic activation, with Aflatoxin B_1

Substance /Dose	S-9: co-factors	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO: ethanol 1:1 200 µl	Dose 6 -	11,388	100	0	0	0	0	0.9 (1)	1.7 (2)	0	0	2.6 (3)
	Dose 5 1:9	9,855	100	0	0	0	2.0 (2)	3.0 (3)	2.0 (2)	0	0	7.1 (7)
	Dose 4 1:3	9,814	100	0	0	1.0 (1)	0	1.0 (1)	0	0	0	2.0 (2)
Aflatoxin B_1 200 µg	Dose 3 -	14,264	125	1.4 (2)	0	0	0	0.7 (1)	0.7 (1)	0	1.4 (2)	2.8 (4)
	Dose 2 1:9	13,891	141	0.7 (1)	0	0.7 (1)	0	0.7 (1)	0.7 (1)	1.4 (2)	0.7 (1)	4.3 (6)
	Dose 1 1:3	14,202	145	1.4 (2)	0	0	0	2.1 (3)	0	0	1.4 (2)	3.5 (5)

Figures in parentheses are actual number of aberrants counted

DMSO : Dimethylsulphoxide

TABLE 156

Saccharomyces cerevisiae D5 Recombinogenic Activity

Project No.:	410110
Contractor:	US Army
Operators:	Jennifer Harvey Colin Riach
Substance:	Cyclophosphamide
Incubation time:	30 min
Activation:	Aroclor-induced Fischer rat
Liver prep. date:	11 April 1979
Date plated:	18 April 1979
Date counted:	27 April 1979
Plates (batch):	M93003/P43142

TABLE 156 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with metabolic activation with cyclophosphamide

Substance /Dose	S9: co-factors	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
PO4 buffer 500 µl	1:9	11,337	100	0	0	0	0	0	0	0	0	0
PO4 buffer 500 µl	1:3	10,978	97	0	0.9 (1)	0	0	0.9 (1)	0	0.9 (1)	0.9 (1)	2.7 (3)
cyclo-phospha-mide 20 mg 35.8mM	1:9	10,723	96	0.9 (1)	0.9 (1)	0	0.9 (1)	0.9 (1)	1.9 (2)	1.9 (2)	1.8 (2)	7.5 (8)
	1:3	9,940	88	1.0 (1)	0	0	1.0 (1)	1.0 (1)	2.0 (2)	0	1.0 (1)	5.0 (5)
cyclo-phospha-mide 10 mg 17.9mM	1:9	11,452	101	0	0.8 (1)	0	0.8 (1)	0	2.6 (3)	0	0.8 (1)	4.2 (5)
	1:3	9,916	87	0	0	1.0 (1)	1.0 (1)	2.0 (2)	1.0 (1)	0	0	5.0 (5)

Figures in parentheses are actual number of aberrants counted

TABLE 157

Saccharomyces cerevisiae D5 Recombinogenic Activity

Project No:	410110
Contractor:	U.S. Army
Operators:	Rowan Hastwell Jennifer Harvey Colin Riach
Substance:	Cyclophosphamide
Incubation time:	30 min
Activation:	Aroclor-induced Fischer Rat
Liver prep. date:	11 April 1979
Date plated:	27 April 1979
Date counted:	9 May 1979
Plates (batch):	R80411

TABLE 157 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity with activation, with Cyclophosphamide

Substance /Dose	S-9 co-factors	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
DMSO 200 µl	1:9	11,335	100	0	0	0	0.9 (1)	0	0	0.9 (1)	0	1.8 (2)
DMSO 200 µl	1:3	12,869	113	0	0	0.8 (1)	0	0	0.8 (1)	1.5 (2)	0	3.1 (4)
Cyclophosphamide 40 mg	1.9	13,456	119	0	0	0.7 (1)	0.7 (1)	0	0	1.5 (2)	0	3.0 (4)
	1.3	12,860	113	0.8 (1)	0	0	0.8 (1)	0	0.8 (1)	0	0.8 (1)	2.3 (3)
Cyclophosphamide 60 mg	1.9	12,764	113	0	0	0	0.8 (1)	0.8 (1)	0	0	0	1.6 (2)
	1.3	13,277	117	1.5 (2)	0	0	0	0	0.8 (1)	0	1.5 (2)	2.3 (3)
Cyclophosphamide 80 mg	1.9	13,533	119	2.2 (3)	0	0	0	0	0	0.7 (1)	2.2 (3)	3.0 (4)
	1.3	13,690	121	0	0	0	0.7 (1)	0	0	0.7 (1)	0	1.5 (2)
Cyclophosphamide 100 mg	1.9	12,806	113	0	0	0	0	0.8 (1)	0	0	0	0.8 (1)
	1.3	13,616	120	0	0	1.5 (2)	0.7 (1)	0	0	0.7 (1)	0	2.9 (4)

Figures in parentheses are actual number of aberrants counted

DMSO = Dimethylsulphoxide

TABLE 158

Saccharomyces cerevisiae D5 Recombination, with activation, modified incubation

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Cyclophosphamide
Incubation time:	18 h
Date plated:	5 June 1979
Date counted:	15 June 1979
Plates (batch):	M53140, M02246
Liver prep. date:	18 May 1979

TABLE 158 (continued)

Saccharomyces cerevisiae D5 Recombination, with activation, modified incubation

Substance	Dose	No. survivors	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Phosphate buffer	Dose 7 -ve control 100µl	13,007	100.0	0	0	0.8 (1)	0.8 (1)	0.8 (1)	0	3.1 (4)	0	5.4 (7)
Cyclophosphamide	Dose 6 1.01 mg	12,543	96.4	0.8 (1)	0	2.4 (3)	1.6 (2)	0.8 (1)	0.8 (1)	4.8 (6)	0.8 (1)	11.2 (14)
	Dose 5 2.03 mg	14,863	114.3	2.0 (3)	2.0 (3)	2.7 (4)	0.7 (1)	3.4 (5)	0	3.4 (5)	4.0 (6)	14.1 (21)
	Dose 4 4.05 mg	14,082	108.3	1.4 (2)	0.7 (1)	1.4 (2)	0.7 (1)	3.6 (5)	0.7 (1)	5.0 (7)	2.1 (3)	13.5 (19)
	Dose 3 8.1 mg	11,721	90.1	4.3 (5)	3.4 (4)	4.3 (5)	0.9 (1)	10.2 (12)	0.9 (1)	6.8 (8)	7.7 (9)	30.7 (36)
	Dose 2 16.2 mg	11,631	89.4	7.7 (9)	3.4 (4)	1.7 (2)	0	6.0 (7)	0.9 (1)	6.0 (7)	11.1 (13)	25.8 (30)
	Dose 1 32.4 mg	12,926	99.4	11.6 (15)	8.5 (11)	3.1 (4)	0.8 (1)	12.4 (16)	1.5 (2)	16.2 (21)	20.1 (26)	54.2 (70)

Figures in parentheses are actual number of aberrants counted

TABLE 159

Saccharomyces cerevisiae D5 Recombinogenic Activity
with and without metabolic activation, with Cyclophosphamide

Project No:	410110
Contractor:	US Army
Operators:	Colin Riach Jennifer Harvey
Substance:	Cyclophosphamide
Incubation time:	18 h (modified method)
Activation:	Aroclor-induced Fischer rat
Liver prep. date:	18 June 1979
Date plated:	26 July 1979
Date counted:	5 July 1979
Plates (batch):	R60541

TABLE 159 (continued)

Estimation of cell numbers (All counts are approximate and are visible not viable count)

Visible count before incubation of stock culture (i.e. using counting chamber) = 1×10^7 cells/ml

Viable count after 18 h incubation in all incubation tubes (i.e. using counting chamber) = 1×10^8 cells/ml

Dilution of incubation mix for plating:

A	1×10^8/ml	0.1 ml A + 9.9 ml phosphate buffer → B
B	1×10^6/ml	0.1 ml B + 9.9 ml phosphate buffer → C
C	1×10^4/ml	3 ml C + 12 ml phosphate buffer → D
D	2×10^3/ml	

NADP "S-9" =

NADP di Na salt	10 mg/ml
G-6-P di Na salt	27.65 mg/ml
$MgCl_2.6H_2O$	1.62 mg/ml
KCl	2.46 mg/ml

Co-factor solution made up in sterile, cold 0.05M - phosphate buffer, pH 7.4, filter sterilised and mixed in the ratio 1 part liver preparation to 9 parts co-factor solution.

TABLE 159 (continued)

NADPH "S-9" =	NADPH tetra Na salt	10 mg/ml
	G-6-P di Na salt	27.65 mg/ml
	$MgCl_2.6H_2O$	1.62 mg/ml
	KCl	2.46 mg/ml

Co-factor solution made up in sterile, cold 0.05M-phosphate buffer, pH 7.4, filter sterilised and mixed in the ratio 1 part liver preparation to 9 parts co-factor solution.

TABLE 159 (continued)

Saccharomyces cerevisiae D5 Recombinogenic Activity
with and without metabolic activation with cyclophosphamide

Substance /Dose	S-9 mix	No. of Survivors: viable count after 18h incubation	% survival	No. of aberrants/10^4 survivors							Frequency of mitotic recombination i.e. red-pink + red-pink-white / total no. colonies calculated as per 10^4 survivors	Total aberrant frequency i.e. total no. aberrants / total no. colonies calculated as per 10^4 survivors
				Pink-red	Pink-red-white	Pink	Red	White-pink	White-red	Hairline		
Phosphate buffer 100 µl	Dose 9 w/o S-9	13,624: $6.6x10^7$/ml	100	0	0.8 (1)	0	0.8 (1)	0.8 (1)	1.5 (2)	1.5 (2)	0.8 (1)	5.3 (7)
	Dose 8 NADP "S-9"	12,311: $6.1x10^7$/ml	100	0	3.2 (4)	1.6 (2)	0.8 (1)	6.5 (8)	1.6 (2)	0.8 (1)	3.2 (4)	14.6 (18)
	Dose 7 NADPH "S-9"	11,836: $5.9x10^7$/ml	100	0	1.7 (2)	0.8 (1)	1.7 (2)	0	0.8 (1)	0.8 (1)	1.7 (2)	5.9 (7)
Cyclophosphamide 20.0 mg 35.8 mM	Dose 6 w/o "S-9"	11,013: $5.5x10^7$/ml	83	1.8 (2)	3.6 (4)	2.7 (3)	0	4.5 (5)	1.8 (2)	1.8 (2)	5.4 (6)	16.3 (18)
	Dose 5 NADP "S-9"	11,005: $5.5x10^7$/ml	90	6.3 (7)	9.0 (10)	1.8 (2)	0	6.3 (7)	4.5 (5)	2.7 (3)	15.4 (17)	30.9 (34)
	Dose 4 NADPH "S-9"	9,964: $5.0x10^7$/ml	85	4.0 (4)	10.0 (10)	2.0 (2)	0	5.0 (5)	2.0 (2)	2.0 (2)	14.1 (14)	25.1 (25)
Cyclophosphamide 40.0 mg 71.6 mM	Dose 3 w/o "S-9"	13,596: $6.7x10^7$/ml	102	1.5 (2)	2.9 (4)	1.5 (2)	3.7 (5)	3.7 (5)	1.5 (2)	1.5 (2)	4.4 (6)	16.2 (22)
	Dose 2 NADP "S-9"	9,896: $4.9x10^7$/ml	80	8.0 (8)	9.1 (9)	0	1.0 (1)	9.1 (9)	5.1 (5)	2.0 (2)	17.2 (17)	34.4 (34)
	Dose 1 NADPH "S-9"	11,576: $5.8x10^7$/ml	98	4.3 (5)	6.9 (8)	0.9 (1)	2.6 (3)	5.2 (6)	1.7 (2)	1.7 (2)	11.2 (13)	23.3 (27)

Figures in parentheses are actual number of aberrants counted

REPORT DISTRIBUTION LIST

25 Copies	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBG Fort Detrick, Frederick, MD 21701
4 Copies	HGDA (SGRD-AJ) Fort Detrick Frederick, MD 21701
12 Copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDN Cameron Station Alexandria, Virginia 22314
1 Copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014
1 Copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 Copy	Librarian US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBD-A Fort Detrick Frederick, MD 21701