

8EHQ-1298-14333

PE Biosystems

A DIVISION OF PERKIN-ELMER

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December 8, 1998

VIA FEDERAL EXPRESS

MR 13213

Document Processing Center 7407
Room G-99, East Tower
(Attn: Section 8(e) Coordinator)
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460



8899000052

Re: Section 8(e) Submittals for HATU and HOAT

Dear Section 8(e) Coordinator:

In accordance with the requirements of Section 8(e) of the Toxic Substances Control Act (TSCA), 15 U.S.C. § 2607(e), PE Biosystems, a division of the Perkin-Elmer Corporation, is submitting information to the U.S. Environmental Protection Agency (EPA) concerning the health effects of two chemical substances that are or have been manufactured, imported, processed and distributed by PE Biosystems and its subsidiaries. These chemical substances are intended for use only in research and development applications.

According to our analysis of available EPA policy and guidance on Section 8(e), it appears that the attached information may not pose a "substantial risk" to human health or the environment, and, thus may not be reportable under Section 8(e). However, given the ambiguity of the Section 8(e) requirements as they apply to the attached information, PE Biosystems is reporting this information pursuant to Section 8(e) in an abundance of caution and with a desire that EPA have all information that it may need to evaluate the risks of chemical products.

I received the attached information on November 17, 1998. Accordingly, I am submitting this information within the 15 working days as required by TSCA Section 8(e).

Identity of Chemical Substances

PE Biosystems is submitting separate sets of information regarding the following two chemical substances:

- 1) 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU)

CAS Number: 148893-10-1

- 2) 1-Hydroxy-7-azabenzotriazole (HOAT)

CAS Number: 39968-33-7

Contains No CBI

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Both of these chemicals are used as activators in peptide and peptide nucleic acid (PNA) synthesis, which are used by academic, governmental and industrial scientists to conduct various types of research.

Name and Address of Person and Company Reporting

This information is being submitted on behalf of PE Biosystems, by:

Debora Van der Sluis
Chemical Regulatory Compliance Manager
PE Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
Telephone: (650) 638-5277
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Summary of Adverse Effects and Source of Information

HATU

Attached at Tab 1 is a report, entitled "O-7-azabenzotriazol-1-YL)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HATU), Magnusson-Kligman Maximisation Test in Guinea Pigs," prepared by Inveresk Research in Scotland (the HATU Sensitization Study). This study was conducted for a PE Biosystems subsidiary (PE Applied Biosystems) in Warrington, England.

The HATU Sensitization Study evaluated the delayed contact hypersensitivity of HATU by means of a Magnusson-Kligman Maximisation Test in guinea pigs. Under the study, researchers exposed 10 guinea pigs to concentrations of 0.5% HATU via intradermal injection ✓ and 75% HATU via topical application. The study produced a result of 10 positive responses. Accordingly, the study concluded that HATU is a sensitizer in guinea pigs.

I have also received information indicating that four employees of PerSeptive Biosystems (a subsidiary of PE Biosystems) and six employees of a company named Proligo Biochemie GmbH of Hamburg, Germany (a former PerSeptive Biosystems manufacturing operation), which also manufactured HATU, have reported the following symptoms which they believe may be associated with exposure to HATU:

Rashes, "red face," respiratory congestion, difficulty breathing, regional swelling, coughing, eye irritation and swelling, headache, allergic reaction, skin irritation, itching, sneezing, severe trouble breathing, asthma, severe dyspnea, cephalgia, rhinorea, obstruction of bronchial airways, permanent fine whistle of bronchial airways, flatulence, and flu-like symptoms.

Some employees have sought medical treatment for these symptoms.

Attached at Tab 2 is a Material Safety Data Sheet (MSDS) that has been revised, effective today, to reflect the information contained in the HATU Sensitization Study and the

employee reports. PE Biosystems is in the process of finalizing (i.e., putting on Company formatted, letterhead paper) and distributing this revised MSDS to its employees and customers. ✓

HOAT

Attached at Tab 3 is a report, entitled "1-Hydroxy-7-azabenzotriazole (HOAT), Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2uvrA," prepared by Inveresk Research in Scotland (the HOAT Mutagenicity Study). This study was prepared for a PE Biosystems subsidiary (PE Applied Biosystems) in Warrington, England.

The HOAT Mutagenicity Study evaluated the mutagenic activity of HOAT in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2uvrA at concentrations ranging from 17 to 5000 µg per plate. The study concluded that, with the exception of *Salmonella typhimurium* strain TA 1537, HOAT was mutagenic to these strains of *Salmonella typhimurium* and *Escherichia coli*, when tested in dimethylsulphoxide up to a predetermined maximum limit (5000 µg per plate).

In addition, I have received information indicating that preliminary results of a mouse lymphoma study also suggest mutagenic activity, albeit weak, of HOAT. I have not yet received the final report of this study, but will submit it to EPA within 15 working days of receiving it.

PE Biosystems is currently evaluating the need to revise the MSDS for HOAT. The company will evaluate the final results of the mouse lymphoma study, and, if necessary, revise the HOAT MSDS to reflect possible mutagenic effects associated with HOAT. If a revised MSDS is prepared, I will submit a copy of this revised MSDS to EPA promptly after revision.

* * * * *

Please contact me at 650-638-5277 if you have any questions about this submittal.

Sincerely,

Debora Van der Sluis

Debora Van der Sluis

Enclosures



CONFIDENTIAL

Inveresk Report Number 16702

**O-(7-azabenzotriazol-1-yl)-1,1,3,3-
tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs**

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Final Page of Report: 34

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22 OCT 1998



Inveresk Research



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Authentication

I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the OECD principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.

E Donald BSc
Study Director

Date



Quality Assurance Statement

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a predetermined schedule.

<u>Date of QA Inspection</u>	<u>Phase</u>	<u>Date of Report to Management</u>
12 August 1998	Dosing (Intradermal)	13 August 1998
16 September 1998	Assessment	17 September 1998
24 September 1998	Dose Preparation	25 September 1998

This report has been audited by Inveresk Research Quality Assurance Personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

Signed: _____ Date: _____
(Quality Assurance)



Personnel Involved

Study Director: E Donald BSc

Senior Technician: E Alves

Quality Assurance: J M Buchanan BSc
D M Vieth BSc



1 Summary

The delayed contact hypersensitivity of a test material, O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) was investigated by means of a Magnusson-Kligman Maximisation Test in guinea pigs.

The Magnusson-Kligman Maximisation Test comprises 2 procedures: an induction and a challenge procedure. The induction procedure consisted of exposure to the test material *via* 2 routes, intradermal injection and topical application. The animals were also exposed to an adjuvant material *via* intradermal injection. This was followed by a challenge exposure to the test material *via* topical application.

Two groups of animals were used in the study, a Test and a Control Group. The Test Group contained 10 animals and the Control Group contained 5. During the induction procedure the Test Group was exposed to 0.5% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) *via* intradermal injection and 75% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) *via* topical application. Both concentrations were the maximum practicable. The Control Group was exposed to vehicle, 0.5% carboxymethylcellulose (CMC) only during this procedure.

Following challenge with 75% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU), 10 (100%) positive responses were noted in the Test Group. No positive responses were noted in the Control Group animals.

Under the conditions of the study, O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) is considered to be a sensitiser in guinea pigs.



2 Introduction

Perkin Elmer Applied Biosystems required information on the delayed contact hypersensitivity of a test material, O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU).

This report gives details of a Magnusson-Kligman Maximisation Test in guinea pigs designed to be compliant with the following guidelines:

OECD Guideline No. 406, Skin Sensitisation, July 1992
EC Directive 92/69/EEC, Annex V, Method B6, Skin Sensitisation, July 1992

The objective of the test was to determine the delayed contact hypersensitivity potential of the test material on guinea pigs.

Skin contact has been defined as a possible route of human exposure and the guinea pig is regarded as a good model for prediction of likely human sensitisers. The Magnusson-Kligman Maximisation Test is one of the methods recommended by current regulatory guidelines.

The test was performed at Elphinstone Research Centre, Inveresk Research.

The study schedule was as follows:

Study Initiation:	10 June 1998
Experimental Start Date:	12 June 1998
Experimental Completion Date:	13 July 1998
Study Completion Date:	See Authentication page for date of Study Director's signature

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of Inveresk Research for 5 years (or for such shorter period of time as, in the opinion of Inveresk, the quality of the material affords evaluation) after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the disposal, transfer or continued storage of raw data.



3 Experimental Procedure

3.1 Test Material

Two containers with a total weight of approximately 50 g (approximate gross weight 100 g) of the test material, O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) were received at Inveresk on 10 June 1998. The test material, a white powder, was stored in the dark at +4°C.

3.1.1 Test Material Formulation

Dosing formulations were prepared on the days of administration to the animals.

The appropriate amount of O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) was weighed and added to the requisite amount of vehicle, 0.5% carboxymethylcellulose (CMC). The formulations were mixed both manually and mechanically until visibly homogeneous.

Vehicle formulations (without test material) were prepared as a control.

3.1.2 Formulation Analysis

Analysis of the formulations was not undertaken.

3.1.3 Other Materials Used on Study

Freund's Complete Adjuvant (FCA) (Difco Batch No. 110367LA) was prepared as a 50% v/v emulsion by adding the requisite amount of FCA to the appropriate amount of sterile distilled water.

Sodium lauryl sulphate (SLS) (Fisons Laboratory Reagent - Batch 23H0311) was prepared as a 100 mg.ml⁻¹ (10%) solution by adding the requisite amount of material to the appropriate amount of sterile distilled water.

3.2 Location of Study

The study was conducted in Room P14 of the Toxicology Accommodation of Inveresk Research.



3.3 *Animals and Management*

Twenty two young adult (less than one year old), nulliparous and non-pregnant, female albino guinea pigs of the Dunkin-Hartley strain, within the weight range 323-372 g on arrival, were used. They were supplied by David Hall Limited, Darley Oaks, Newchurch, Burton-on-Trent, Staffordshire and arrived at Elphinstone Research Centre on 5 June 1998.

3.3.1 *Acclimatisation*

The animals were allowed to acclimatise for at least 7 days prior to commencement of the study.

3.3.2 *Allocation to Dose Groups*

No formal randomisation procedure was applied. Animals were allocated to dose group by introduction to cages pre-labelled with study number, animal number and group.

3.3.3 *Animal Identification*

The animals were identified individually within each cage by means of a colour code and an indelible ear mark. The colour code represented the group of the animal and the ear mark individually identified the animals within each cage.

3.3.4 *Room Environment*

The animals were multiply housed in aluminium cages (dimensions 48 x 61 x 25 cm) with a grid floor beneath which was an absorbent paper lined tray.

The dose ranging animals were housed 2 or 3 to a cage and the main study animals 5 to a cage.

Each cage was supplied with a water bottle and a food hopper.

Mean environmental maximum and minimum temperatures were 21°C and 20°C and mean relative humidity was 54%. A 12 h light/dark cycle was in operation (light hours 0700-1900 h) with a minimum of 15 air changes per hour.

3.3.5 *Room Sanitation*

The floor, cages, cage racks, walls and ceiling were cleaned with disinfectant solution (0.5% Tego 2000, Th. Goldschmidt and Company Limited, Middlesex, England) as necessary.



3.3.6 Diet and Water Supply

Guinea Pig Diet FD1, supplied by Special Diets Services Ltd, 1 Stepfield, Witham, Essex, CM8 3AD was available *ad libitum*. The diet was supplied with a typical batch analysis for major nutritive constituents and significant contaminants. A typical analytical certificate is presented in Appendix 1. Each cage was also offered a supplement of organically grown cabbage *ca* twice weekly.

Domestic mains quality water was available *ad libitum* throughout the study. The water used by Inveresk laboratories is analysed, by the local water authority, at 6 month intervals for dissolved materials, heavy metals, pesticide residues, pH, nitrates and nitrites. The certificate for the analysis conducted most recently is presented in Appendix 2.

The food and water are considered not to contain any additional substances in sufficient concentration to have any influence on the outcome of the study.

3.4 Treatment

3.4.1 Dose Groups

Animal were allocated to dose groups as follows:

Group	Number of Animals
<u>Dose Ranging</u>	
Dose Ranging for Induction – Intradermal Injection	3
Dose Ranging for Induction – Topical Application	2
Dose Ranging for Challenge	2
<u>Main Study</u>	
Control Group	5
Test Group	10

3.4.2 Justification of Dose Levels

3.4.2.1 Dose Ranging for Induction

Dose levels for dose ranging for induction were a range of 8 concentrations of test material and took account of the maximum practicable concentration for administration.

3.4.2.2 Induction

Dose levels for induction were selected based on the dose ranging for induction and were such that necrosis or severe reactions were not produced. The levels selected were the highest to cause mild to moderate skin irritation and were also well tolerated systemically.



3.4.2.3 Dose Ranging for Challenge

Dose levels for dose ranging for challenge were selected based on the dose ranging for induction results and the topical induction results. These were a range of 4 levels of the test material and were selected to determine the maximum non-irritant concentration at challenge.

3.4.2.4 Challenge

Dose levels for challenge were selected based on the dose ranging for challenge results and were the highest concentration that did not cause irritation.

3.4.3 Route and Duration of Administration

3.4.3.1 Dose Ranging for Induction

Dose ranging for induction was conducted via intradermal injections and topical application of different concentrations of the test material.

3.4.3.1.1 Intradermal Injections

On the day prior to administration, hair was clipped from an area (4 cm x 6 cm) across the scapular region of the animals.

On the following day, each animal was treated with 4 different concentrations of the test material injected intradermally as follows:

Number of Animals	Dosing Formulation	Concentration (% HATU)	Volume (ml)	Region of Scapula
1	A	75*	0.1	Upper Left
	B	50*	0.1	Upper Right
	C	25*	0.1	Lower Left
	D	10*	0.1	Lower Right
2	E	5*	0.1	Upper Left
	F	2*	0.1	Upper Right
	G	0.5	0.1	Lower Left
	H	0.1	0.1	Lower Right

* = Unable or very difficult to inject

3.4.3.1.2 Topical Application

On the day prior to administration, hair was clipped from an area (4 cm x 6 cm) on both flanks of the animals.

On the following day, each animal was treated with 4 different concentrations of the test material applied topically under webriil patches (2.5 cm x 2.5 cm) as follows:



Number of Animals	Dosing Formulation	Concentration (% HATU)	Volume (ml)	Region of Flank
2	A	75	0.5	Upper Left
	B	50	0.5	Upper Right
	C	25	0.5	Lower Left
	D	10	0.5	Lower Right

Each patch was covered with aluminium foil and then with Blenderm occlusive tape and elastic bandage wrapped round the torso of the animal.

The patches were removed after 48 h and then the test site wiped with sterile water. Dosing formulations A, B and C were still evident after wiping.

3.4.3.2 Main Study Induction: Intradermal Injections

On the day prior to administration, hair was clipped from an area (4 cm x 6 cm) across the scapular region of the animals.

On the following day, each animal was treated with 6 intradermal injections as follows:

Group	Injection	Treatment	Volume (ml)	Region of Scapula
Control	1	50% aqueous FCA	0.1	Upper Left
	2	50% aqueous FCA	0.1	Upper Right
	3	0.5% CMC (vehicle)	0.1	Mid Left
	4	0.5% CMC	0.1	Mid Right
	5	0.5% CMC:50% aqueous FCA (1:1)	0.1	Lower Left
	6	0.5% CMC:50% aqueous FCA (1:1)	0.1	Lower Right
Test	1	50% aqueous FCA	0.1	Upper Left
	2	50% aqueous FCA	0.1	Upper Right
	3	1% HATU in 0.5% CMC:0.5% CMC (1:1)*	0.1	Mid Left
	4	1% HATU in 0.5% CMC:0.5% CMC (1:1)*	0.1	Mid Right
	5	1% HATU in 0.5% CMC:50% aqueous FCA (1:1)*	0.1	Lower Left
	6	1% HATU in 0.5% CMC:50% aqueous FCA (1:1)*	0.1	Lower Right

* = Final concentration of HATU was 0.5%



3.4.3.3 Main Study: Topical Application

Six days after the intradermal injections, the hair was clipped from an area (4 cm x 6 cm) across the scapular region of the animals. Based on the dose ranging results it was necessary to treat the clipped area with 0.5 ml of 10% sodium lauryl sulphate (SLS).

On the following day, each animal was treated with 1 topical application to the scapular region under a webril patch (2 cm x 4 cm) as follows:

Group	Pre-treated with SLS	Treatment	Concentration (% HATU)	Volume (ml)
Control	Yes	0.5% CMC	0	0.5
Test	Yes	HATU	75	0.5

Each patch was covered with aluminium foil and then with Blenderm occlusive tape and elastic bandage wrapped round the torso of the animal.

The patches were removed after 48 h and then the test site wiped with sterile water and delineated.

3.4.3.4 Dose Ranging for Challenge

Twenty two days prior to administration of the test material, hair was clipped from an area (4 cm x 6 cm) across the scapular region of the dose ranging animals.

On the following day, each animal was treated with 6 intradermal injections as follows:

Injection	Treatment	Volume (ml)	Region of Scapula
1	50% aqueous FCA	0.1	Upper Left
2	50% aqueous FCA	0.1	Upper Right
3	0.5% CMC (vehicle)	0.1	Mid Left
4	0.5% CMC	0.1	Mid Right
5	0.5% CMC:50% aqueous FCA (1:1)	0.1	Lower Left
6	0.5% CMC:50% aqueous FCA (1:1)	0.1	Lower Right

Six days later, hair was clipped from an area (4 cm x 6 cm) across the scapular region of the 2 guinea pigs. Based on the dose ranging results it was necessary to treat the clipped area with 0.5 ml of 10% SLS.

On the following day, each animal was treated with 1 topical application to the scapular region under a webril patch (2 cm x 4 cm) as follows:

Number of Animals	Pre-treated with SLS	Treatment	Volume (ml)
2	Yes	0.5% CMC	0.5



Each patch was covered with aluminium foil and then with Blenderm occlusive tape and elastic bandage wrapped round the torso of the animal.

The patches were removed after 48 h and then the test site wiped with sterile water.

Eleven days later, one day prior to test material administration, hair was clipped from an area (4 cm x 6 cm) on both flanks of the 2 guinea pigs.

On the following day, each animal was treated with 4 different concentrations of the test material applied topically to the flanks under webril patches (2.5 cm x 2.5 cm) as follows:

Number of Animals	Dosing Formulation	Concentration (% HATU)	Volume (ml)	Region of Flank
2	A	75	0.5	Upper Left
	B	50	0.5	Lower Left
	C	25	0.5	Upper Right
	D	10	0.5	Lower Right

Each patch was covered with aluminium foil and then with Blenderm occlusive tape and elastic bandage wrapped round the torso of the animal.

The patches were removed after 24 h and then the test site wiped with sterile water. Test sites were also clipped following patch removal and delineated.

3.4.3.5 Main Study: Challenge

Thirteen days after topical application, hair was clipped from an area (5 cm x 5 cm) on both flanks of the main study animals.

On the following day, each animal was treated with test material and vehicle applied topically to the flanks under 2 webril patches (2.5 cm x 2.5 cm) as follows:

Group	Dosing Formulation	Concentration (% HATU)	Volume (ml)	Region of Flank
Control	A	0	0.5	Upper Right
	B	75	0.5	Upper Left
Test	A	0	0.5	Upper Right
	B	75	0.5	Upper Left

Each patch was covered with aluminium foil and then with Blenderm occlusive tape and elastic bandage wrapped round the torso of the animal.

The patches were removed after 24 h and then the test site wiped with 0.5% CMC. Test sites were also clipped following patch removal and delineated.



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3.5 Observations

3.5.1 Viability

All the animals were checked for viability early in the morning and again as late as possible on each day.

3.5.2 Clinical Observations

All the animals were examined for reaction to treatment. The onset, intensity and duration of any signs were recorded.

3.5.3 Test Site Observations

Test sites were examined and skin reactions were graded according to the scoring system detailed in Appendix 3. The timing of the test site observations was as follows:

Phase of Study	Time after Injection/Patch Removal
<u>Dose Ranging for Induction</u>	
Intradermal Injection	24 h, 48 h, 72 h
Topical Application	24 h, 48 h
<u>Dose Ranging for Challenge</u>	
Intradermal Injection	24 h, 48 h, 72 h
Topical Application	24 h, 48 h
Challenge	24 h, 48 h
<u>Main Study</u>	
Induction - Intradermal Injection	24 h
Induction - Topical Application	24 h
Challenge	24 h, 48 h

3.5.4 Body Weights

Body weights were recorded for each main study animal only.

The body weight of each individual animal was recorded on the day prior to commencement of the induction procedure and as soon as practicable after the final test site observation at challenge.

3.6 Terminal Studies

3.6.1 Animals Used

All animals.



3.6.2 Timing

As soon as practicable, or after the final test site observation at challenge.

3.6.3 Method of Sacrifice

The animals were sacrificed by exposure to carbon dioxide and discarded without necropsy.

3.7 Positive Control

The sensitivity of the Dunkin-Hartley strain of guinea pig to a mild/moderate sensitiser, 2-mercaptobenzothiazole (MBT), is checked at 6 month intervals. In the most recent positive control test, completed on 13 July 1998, MBT was applied at induction at concentrations of 5% (injection) and 25% (topical) w/v in maize oil. Challenge was carried out at a concentration of 25% MBT in maize oil.



4 Results

4.1 Dose Ranging for Induction

Reaction scores are presented in Table 1.

4.1.1 Intradermal Injections

Concentrations from 2 to 75% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) were uninjectable. Discrete to moderate erythema were noted at 0.5 and 0.1% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU).

4.1.2 Topical Application

At concentrations of up to 75% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU), no reactions were noted in any animal.

Based on these results, a dose level of 1% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) (final concentration 0.5%) was selected for the intradermal induction and a concentration of 75% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) was selected for topical induction.

4.2 Main Study Induction

4.2.1 Intradermal Injections

Reaction scores are presented in Table 2.

Slight erythema was noted in all animals.

4.2.2 Topical Application

Reaction scores are presented in Table 3.

Slight erythema was noted in all animals. Additionally, the skin was hard, cracked and scabbed at the test site all animals.

4.3 Dose Ranging for Challenge

Reaction scores are presented in Table 4.

4.3.1 Intradermal Injections

Slight erythema was noted in both animals.

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4.3.2 Topical Application

No erythemic reactions were noted in either animal. Both test sites were noted to be flaky, dry, hard and scabbed.

4.3.3 Challenge

No reactions were noted in either animal.

Based on this, 75% O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluronium hexafluorophosphate (HATU) was selected for the challenge phase.

4.4 Main Study Challenge

Reaction scores are presented in Table 5.

Following challenge, ten (100 %) positive responses were noted in the Test Group animals. No positive responses were noted in the Control Group animals.

4.5 Body Weights

Individual body weights and gains are presented in Table 6.

Body weight performance was considered to have been satisfactory.

4.6 Positive Control

Details of the positive control results are presented in Appendices 4-6.

The most recent positive control was completed on 13 July 1998. Following challenge with MBT at a dose level of 25% w/v in maize oil, 100% of the Test Group animals and none of the Control Group animals reacted positively.

These results demonstrate the ability of the test method to identify a mild/moderate sensitiser.



5 **Conclusion**

Under the conditions of the study, a polar extract of O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU) is considered to be a sensitiser in guinea pigs.

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Test
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Test



6 *Reference*

Magnusson B and Kligman A M (1969), The identification of contact allergens by animal assay. The guinea pig maximisation test. *J Invest Dermat*, 52, 268-276.



7 Tables

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O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosate (HATU)
 Magnusson-Kligman Maximisation Test in Guinea Pigs
 Dose Ranging for Induction
 Reaction Scores

Table 1

Intradermal Injection

Concentration (% HATU)	Cage Number/Animal Number/Time after Injection								
	4								
	1			3			4		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
75	*	*	*	-	-	-	-	-	-
50	*	*	*	-	-	-	-	-	-
25	*	*	*	-	-	-	-	-	-
10	*	*	*	-	-	-	-	-	-
5	-	-	-	-	-	-	0**	1**	1**
2	-	-	-	-	-	-	0**	0**	0**
0.5	-	-	-	2	1	1	1	1	1
0.1	-	-	-	1	1	1	1	1	1

* = Unable to inject

** = Small amount injected

Topical Application

Concentration (% HATU)	Cage Number/Animal Number/Time after Patch Removal			
	5			
	1		2	
	24 h	48 h	24 h	48 h
75∅	0	0	0	0
50∅	0	0	0	0
25∅	0	0	0	0
10	0	0	0	0

∅ = Test material still evident after wiping

Scoring system is detailed in Appendix 3



***O*-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
 Magnusson-Kligman Maximisation Test in Guinea Pigs
 Intradermal Induction
 Reaction Scores**

Table 2

Group and Concentration (% HATU)	Cage	Animal	Time after Injection
			24 h
Control (0)	1	1	1
		2	1
		3	1
		4	1
		5	1
Test (0.5)	2	1	1
		2	1
		3	1
		4	1
		5	1
	3	1	1
		2	1
		3	1
		4	1
		5	1

Scoring system is detailed in Appendix 3



**O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
 Magnusson-Kligman Maximisation Test in Guinea Pigs
 Topical Application
 Reaction Scores**

Table 3

Group and Concentration (% HATU)	Cage	Animal	Time after Patch Removal
			24 h
Control (0)	1	1	1
		2	1
		3	1
		4	1
		5	1
Test (75)	2	1	1
		2	1
		3	1
		4	1
		5	1
	3	1	1
		2	1
		3	1
		4	1
		5	1

All test sites are hard, cracked and scabbed
 Scoring system is detailed in Appendix 3



***O*-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
 Magnusson-Kligman Maximisation Test in Guinea Pigs
 Dose Ranging for Challenge
 Reaction Scores**

Table 4

Intradermal Injection

Cage	Animal Number/Time after Injection					
	1			2		
6	24 h	48 h	72 h	24 h	48 h	72 h
		1	1	1	1	1

Topical Application

Cage	Pre-treatment with SLS	Animal Number/Time after Patch Removal			
		1		2	
6	Yes	24 h	48 h	24 h	48 h
				0*	0*

* = Test site flaky and/or dry, hard and scabbed

Challenge

Concentration (% HATU)	Cage Number/Animal Number/Time after Patch Removal			
	6			
	1		2	
	24 h	48 h	24 h	48 h
75	0	0	0	0
50	0	0	0	0
25	0	0	0	0
10	0	0	0	0

Scoring system is detailed in Appendix 3



**O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs
Challenge
Reaction Scores**

Table 5

Group/ Cage	Animal	% HATU/Time after Patch Removal				% HATU/ Response		% HATU/% Positive	
		75		0		75	0	75	0
		24 h	48 h	24 h	48 h				
Control/ 1	1	0	0	0	0	-	-	0	0
	2	0	0	0	0	-	-		
	3	0	0	0	0	-	-		
	4	0	0	0	0	-	-		
	5	0	0	0	0	-	-		
Test/ 2	1	1	0∅	0	0	+	-	100	0
	2	2∅	1∅	0	0	+	-		
	3	1	1∅	0	0	+	-		
	4	2	1∅	0	0	+	-		
	5	1	1∅	0	0	+	-		
Test/ 3	1	1	1	0	0	+	-	100	0
	2	1	1∅	0	0	+	-		
	3	2∅	1∅	0	0	+	-		
	4	1	.	0	.	+	-		
	5	2∅	1∅	0	0	+	-		

∅ = Scabs and/or flaky skin at test site
 * = Animal found dead prior to assessment
 + = Positive
 - = Negative
 Scoring system is detailed in Appendix 3



**O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
 Magnusson-Kligman Maximisation Test in Guinea Pigs
 Body Weights (g)**

Table 6

Group	Cage	Animal	Body Weight (g)		
			Start of Study	End of Study	Gain
Control	1	1	476	583	107
		2	473	665	192
		3	449	595	146
		4	521	737	216
		5	510	700	190
Test	2	1	492	525	33
		2	497	638	141
		3	481	683	202
		4	473	651	178
		5	463	605	142
	3	1	489	612	123
		2	511	665	154
		3	482	579	97
		4	510	*	*
		5	535	638	103

* = Animal found dead before end of study



8 Appendices

O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs
Appendix 1 Typical Analysis of Diet

GUINEA PIG DIET FDI

CALCULATED ANALYSIS

NUTRIENTS			SUPPLEMENTATION AS/IT SUPPLEMENTATION	NUTRIENTS			SUPPLEMENTATION AS/IT SUPPLEMENTATION
Crude Oil	%	3.40	2.4	AMINO ACIDS			
Crude Protein	%	19.00		Glycine	%	1.26	
Crude Fibre	%	11.90		Aspartic Acid	%	1.73	
Ash	%	8.70		Glutamic Acid	%	3.19	
N F E	%	47.30		Proline	%	1.13	
Urea	%			Serine	%	0.87	
Dig Crude Oil	%	3.10		Hydroxyproline	%	0.06	
Dig Crude Protein	%	17.10		Hydroxylysine	%		
Tot Diet Fibre	%	26.90		Alanine	%	0.07	
Pectin	%	2.70		MAJOR MINERALS			
Hemicellulose	%	12.60	Calcium	%	1.15	0.47	
Celulose	%	9.80	Total Phosphorus	%	0.86	0.22	
Lignin	%	1.80	Phytate Phosphorus	%	0.24		
Starches	%	25.90	Available Phosphorus	%	0.62	0.22	
Sugars	%	6.10	Sodium	%	0.28	0.21	
ENERGY			Chlorine	%	0.43	0.32	
Gross Energy	MJ/kg	14.90	Magnesium	%	0.34		
Dig Energy	MJ/kg	9.90	Potassium	%	1.27		
Met Energy	MJ/kg	8.90	TRACE MINERALS				
FATTY ACIDS			Iron	mg/kg	537.0	335.0	
Myristic Acid	%	0.03	Copper	mg/kg	16.0	6.0	
Palmitic Acid	%	0.18	Manganese	mg/kg	173.0	125.0	
Oleic Acid	%	0.70	Zinc	mg/kg	69.0	53.0	
Linoleic Acid	%	0.67	Cobalt	µg/kg	720.0	700.0	
Arachidonic Acid	%	0.48	Iodine	µg/kg	1257.0	700.0	
Cuprenonic Acid	%	0.19	Selenium	µg/kg	281.0		
Lauroic Acid	%	0.04	Fluorine	mg/kg	42.0	22.0	
Myristic Acid	%	0.17	VITAMINS				
Palmitic Acid	%	0.37	Retinol	µg/kg	68891.0	1020.0	
Stearic Acid	%	0.07	Vitamin A	IU/kg	227340.0	3366.0	
AMINO ACIDS			Cholecalciferol	µg/kg	45.0	20.6	
Arginine	%	1.34	Vitamin D ₃	µg/kg	1800.0	824.0	
Lysine	%	0.99	α-Tocopherol	mg/kg	87.0	24.0	
Methionine	%	0.40	Vitamin E	mg/kg	73.7	26.4	
Cysteine	%	0.31	Vitamin B ₁	mg/kg	8.3	3.3	
Tryptophan	%	0.29	Vitamin B ₂	mg/kg	14.2	8.3	
Methionine	%	0.44	Vitamin B ₃	mg/kg	11.4	8.3	
Threonine	%	0.77	Vitamin B ₅	mg/kg	12.6	4.2	
Isoleucine	%	0.83	Vitamin B ₆	mg/kg	2678.0	2500.0	
Leucine	%	1.37	Vitamin C	mg/kg	134.5	41.6	
Phenylalanine	%	0.69	Vitamin K ₁	µg/kg	8.8	8.3	
Valine	%	1.02	Folic Acid	µg/kg	70.9	20.8	
Tyrosine	%	0.64	Nicotinic Acid	mg/kg	30.9	12.3	
Taurine	%		Pantothenic Acid	mg/kg	1821.0	670.0	
			Choline	mg/kg	2110.0		
			Inositol	µg/kg	376.0	85.0	
			Biotin	µg/kg			
			p-Aminobenzoic Acid	mg/kg	148.2		
			β-Carotene	µg/kg			
			PIGMENTS				
			Xanthophyll	mg/kg			

Note 1: All values calculated to nominal 10% moisture content.
Note 2: Values on left are based on moist values.
Note 3: Values on right are presented as dry weight basis.

Note 4: 1 µg Retinol = 33 IU Vitamin A activity.
Note 5: Total Retinol content includes the Retinol equivalent of Carotene.
Note 6: 1 µg β-Carotene = 16 IU Vitamin A activity.

Note 7: 1 µg Cholecalciferol = 40 IU Vitamin D₃ activity.
Note 8: 1 mg α-Tocopherol = 1 IU Vitamin E activity.
Note 9: 1 µg = 226.73 Calcium.



**O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
 Magnusson-Kligman Maximisation Test in Guinea Pigs
 Appendix 2 Typical Analysis of Water as Used on Study**

Water Quality Directorate

Edinburgh Laboratory
 4 Marine Esplanade
 Edinburgh
 EH6 7LU

Tel: 0131 555 7903
 Fax: 0131 555 7979



East of Scotland Water

TEST REPORT

Inveresk Research Int. Ltd.
 Environmental Chemistry
 Tranent
 EH33 2NE

Date of Report: 14/07/98
 Order No.: None.
 Lab. Ref.: WWM/18901
 Cust. Ref.: None.
 Taken on: 03/06/98
 Received on: 04/06/98
 Taken by: B Ewart
 Analysis Started: 04/06/98
 Page: 1 of 2

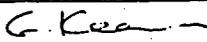
F.A.O.:

Description: Inveresk Research Int.Ltd,Environmental Chemistry,Tranent EH33 2NE.

Test Results:

pH	7.7
Suspended solids	<4 mg/l
Chloride (as Cl)	10 mg/l
Fluoride (as F)	<100 ug/l
Nitrate (as NO ₃)	2.63 mg/l
Sulphate (as SO ₄)	17 mg/l
Alkalinity (as HCO ₃)	54 mg/l
Conductivity	148 uS/cm
Silver (as Ag)	<0.18 ug/l
Arsenic (as As)	0.2 ug/l
Boron (as B)	<17 ug/l
Cadmium (as Cd)	<0.1 ug/l
COD	<25 mg/l
Chromium (as Cr)	<1.7 ug/l
Cyanide (as CN)	<1.00 ug/l
Dry Residues	115.0 mg/l
Total Haloforms	20 ug/l
bromodichloromethane	4.6 ug/l
bromoform	<2.0 ug/l
chloroform	13.6 ug/l
dibromochloromethane	2.0 ug/l
Mercury (as Hg)	<0.05 ug/l
Coliform organisms	None detected in 100ml
E.coli	None detected in 100ml
Plate count at 22C	None detected in 1ml
Plate count at 37C	None detected in 1ml
Salmonella spp.	Not detected
Volume filtered	1 litres
C.Pert	None detected in 100ml
F.Strep	None detected in 100ml
Ammonia (as NH ₄)	<0.02 mg/l
Nickel (as Ni)	1.5 ug/l

Nitrite (as NO ₂)	<0.01 mg/l
Heptachlor	<0.010 ug/l
Trifluralin	<0.010 ug/l
alpha endosulphan	<0.010 ug/l
beta endosulphan	<0.010 ug/l
opDDE	<0.010 ug/l
opDDT	<0.010 ug/l
opTDE	<0.010 ug/l
Aldrin	<0.010 ug/l
Alpha HCH	ug/l
Dieldrin	<0.010 ug/l
Endrin	<0.010 ug/l
Gamma HCH	ug/l
HCB	<0.010 ug/l
ppDDE	<0.010 ug/l
ppDDT	<0.010 ug/l
ppTDE	<0.010 ug/l
Carbophenothion	<0.011 ug/l
Ethyl parathion	<0.011 ug/l
Fenthion	<0.011 ug/l
Methyl parathion	<0.010 ug/l
Azinphos ethyl	<0.021 ug/l
Chlorfenvinphos	<0.011 ug/l
Fenitrothion	<0.010 ug/l
Malathion	<0.010 ug/l
Methyl azinphos	<0.016 ug/l
Benzo(a)pyrene	<0.001 ug/l
Benzo(b)fluoranthene	<0.004 ug/l
Benzo(ghi)perylene	<0.004 ug/l
Benzo(k)fluoranthene	<0.004 ug/l
Fluoranthene	<0.004 ug/l
Indeno(123cd)pyrene	<0.004 ug/l

Authorised By: 
 Name: G KEENAN
 CHEMIST
 Date: 14 JUL 1998


 Appendix 2 Typical Analysis of Water as Used on Study
 (continued)

Lab Ref.: WM/18901

Page 2 of 2

Total PAHs	0.000 ug/l	Sulphide (as S)	0.01 mg/l
Lead (as Pb)	<0.25 ug/l	Total Organic Carbon (as C)	1.4 mg/l
CB 101	<0.010 ug/l	Atrazine	<0.010 ug/l
CB 105	<0.010 ug/l	Propazine	<0.010 ug/l
CB 118	<0.010 ug/l	Simazine	<0.010 ug/l
CB 138	<0.010 ug/l	Trietazine	<0.010 ug/l
CB 149	<0.010 ug/l	Turbidity	<0.1 NTU
CB 153	<0.010 ug/l	1,2 dichloroethane	<1.0 ug/l
CB 180	<0.010 ug/l	1,2,4 trichlorobenzene	<1.0 ug/l
CB 28	<0.010 ug/l	3-chlorotoluene	<1.0 ug/l
CB 31	<0.010 ug/l	Tetrachloroethene	<1.0 ug/l
CB 52	<0.010 ug/l	Tetrachloromethane	<0.3 ug/l
TOTAL	0.000 ug/l	Trichloroethene	<3.0 ug/l
2,4,6 Tri Chlorophenol	<0.05 ug/l	Aluminium (as Al)	9 ug/l
Pentachlorophenol	<0.07 ug/l	Barium (as Ba)	44.8 mg/l
Phenol	<0.05 ug/l	Calcium (as Ca)	16.2 mg/l
Total Cresols (m,p,o)	<0.05 ug/l	Copper (as Cu)	18.3 ug/l
Total Phenols (as C ₆ H ₅ OH)	<0.06 ug/l	Iron (as Fe)	4 ug/l
m Chlorophenol	<0.07 ug/l	Magnesium (as Mg)	5.8 mg/l
o Chlorophenol	<0.06 ug/l	Manganese (as Mn)	1.1 ug/l
Phosphorus SR (as P)	<65 ug/l	Potassium (as K)	0.60 mg/l
Oxidizability (as O)	0.80 mg/l	Sodium (as Na)	6.1 mg/l
Antimony (as Sb)	0.3 ug/l	Zinc (as Zn)	3.8 ug/l
Selenium (as Se)	0.3 ug/l		

Analyst's Remarks: PAH analysis sub-contracted to Stirling *

Analysis Comments:

Signature	G. Keen	Date:
G. KEEN CHIEF ANALYST 14 JUL 1991		



**O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs**

Appendix 3 Scoring System

Assessment of Reactions

Grade

No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3



Appendix 4
O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs
Positive Control: Intradermal Induction
Reaction Scores

Grade

-0
-1
-2
-3

Group and Concentration (% MBT)	Cage	Animal	Time after Injection
			24 h
Control (0)	1	1	1
		2	1
		3	1
		4	1
		5	1
	2	1	1
		2	1
		3	1
		4	1
		5	1
Test (5)	3	1	1
		2	1
		3	1
		4	1
		5	1
	4	1	1
		2	1
		3	1
		4	1
		5	1
	5	1	1
		2	1
		3	1
		4	1
		5	1
	6	1	1
		2	1
		3	1
		4	1
		5	1

Scoring system is detailed in Appendix 3



Appendix 5
O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs
Positive Control: Topical Induction
Reaction Scores

Group and Concentration (% MBT)	Cage	Animal	Time after Patch Removal
			24 h
Control (0)	1	1	0
		2	0
		3	0
		4	0
		5	0
	2	1	0
		2	0
		3	0
		4	0
		5	0
Test (25)	3	1	0
		2	0a
		3	0
		4	0
		5	0
	4	1	0
		2	0
		3	0a
		4	0a
		5	0
	5	1	0
		2	0
		3	0
		4	0
		5	0
6	1	0	
	2	0	
	3	0	
	4	0	
	5	0a	

a = Scabbing evident on test site
 Scoring system is detailed in Appendix 3



O-(7-azabenzotriazol-1-yl)-1,1,1,3-tetramethyluroniumhexafluorophosphate (HATU)
Magnusson-Kligman Maximisation Test in Guinea Pigs
Positive Control: Challenge
Reaction Scores

Appendix 6

Group	Cage	Animal	Concentration (% MBT)/ Time after Patch Removal				Concentration (% MBT)/Response		Concentration (% MBT)/ % Positive	
			25		0		25	0	25	0
			24 h	48 h	24 h	48 h				
Control	1	1	0	0	0	0	-	-	0	0
		2	0	0	0	0	-	-		
		3	0	0	0	0	-	-		
		4	0	0	0	0	-	-		
		5	0	0	0	0	-	-		
	2	1	0	0	0	0	-	-		
		2	0	0	0	0	-	-		
		3	0	0	0	0	-	-		
		4	0	0	0	0	-	-		
		5	0	0	0	0	-	-		
Test	3	1	2	1*	0	0	+	-	100	0
		2	1	1*	0	0	+	-		
		3	1	1	0	0	+	-		
		4	1	1	0	0	+	-		
		5	1	1	0	0	+	-		
	4	1	2	1*	0	0	+	-		
		2	1	1*	0	0	+	-		
		3	2*	1*	0	0	+	-		
		4	2	1*	0	0	+	-		
		5	2	1*	0	0	+	-		
	5	1	1	1	0	0	+	-		
		2	2	1*	0	0	+	-		
		3	1	1	0	0	+	-		
		4	2	1*	0	0	+	-		
		5	1	1	0	0	+	-		
	6	1	1	1	0	0	+	-		
		2	2	2*	0	0	+	-		
		3	2	2*	0	0	+	-		
		4	0	1	0	0	+	-		
		5	3	2*	0	0	+	-		

- = Negative

+ = Positive

* = Flaky and/or scabbing of skin at test site

Scoring system is detailed in Appendix 3

**PE APPLIED BIOSYSTEMS
ATTORNEY-CLIENT PRIVILEGED**

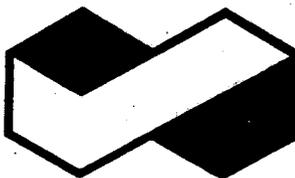
**O-(7-azabenzotriazol-1-yl)-1,1,3,3-
tetramethyluroniumhexafluorophosphate
(HATU)**

**Magnusson-Kligman Maximisation Test
in Guinea Pigs**

Inveresk Project Number 570695

Inveresk Report Number 16702

DRAFT



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BEGIN MSDS 00201050

SECTION 1 CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

P E APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(650) 570-6667 (USA)
01925-825650 (UK)

24 HOUR EMERGENCY RESPONSE NUMBER:
1-800-424-9300 (NORTH AMERICA)
1-703-527-3887 (INTERNATIONAL)

SUBSTANCE: HATU

TRADE NAMES/SYNONYMS:

2-(1H-7-AZABENZOTRIAZOL-1-YL)-1,1,3,3-TETRAMETHYL URONIUM HEXAFLUORO-
PHOSPHATE; METHANAMINIUM,
N-((DIMETHYLAMINO)(3H-1,2,3-TRIAZOLO(4,5-B)PYRIDIN-3-
YLOXY)METHYLENE)-N-METHYL-, HEXAFLUOROPHOSPHATE(1-);
N-((DIMETHYLAMINO)(3H-1,2,3-TRIAZOLO(4,5-B)PYRIDIN-3-YLOXY)METHYLENE)-
N-METHYLMETHANAMINIUM HEXAFLUOROPHOSPHATE(1-);
3H-1,2,3-TRIAZOLO(4,5-B)PYRIDINE, METHANAMINIUM DERIVATIVE; GEN063080;
C10H15F6N6OP; MIB33060; 00201050

CHEMICAL FAMILY: phosphates

CREATION DATE: Sep 28 1993
REVISION DATE: Dec 08 1998

SECTION 2 COMPOSITION, INFORMATION ON INGREDIENTS

COMPONENT: HATU
CAS NUMBER: 148893-10-1
EC NUMBER: Not assigned.
PERCENTAGE: 100.0

SECTION 3 HAZARDS IDENTIFICATION

NFPA RATINGS (SCALE 0-4): HEALTH=2 FIRE=1 REACTIVITY=0

EC CLASSIFICATION (CALCULATED):
Xn Harmful

R 42/43

EMERGENCY OVERVIEW:

COLOR: off-white

PHYSICAL FORM: solid (at 20 degrees C and 101.3 kPa)

MAJOR HEALTH HAZARDS: Proposition 65: N, allergic reactions

PHYSICAL HAZARDS: Dust/air mixtures may ignite or explode.

POTENTIAL HEALTH EFFECTS:

INHALATION:

SHORT TERM EXPOSURE: May cause allergic reactions

LONG TERM EXPOSURE: May cause allergic reactions.

SKIN CONTACT:
 SHORT TERM EXPOSURE: May cause irritation.
 LONG TERM EXPOSURE: May cause allergic reactions., HATU was found to cause skin sensitization in a study in guinea pigs.

EYE CONTACT:
 SHORT TERM EXPOSURE: May cause irritation.
 LONG TERM EXPOSURE: May cause allergic reactions.

INGESTION:
 SHORT TERM EXPOSURE: Acute oral LD50 (rats): greater than 2000 mg/kg.
 LONG TERM EXPOSURE: no information is available

CARCINOGEN STATUS:
 OSHA: N
 NTP: N
 IARC: N

 SECTION 4 FIRST AID MEASURES

INHALATION: Remove from exposure immediately. Use a bag valve mask or similar device to perform artificial respiration (rescue breathing) if needed. Get medical attention.

SKIN CONTACT: Remove contaminated clothing, jewelry, and shoes immediately. Wash with soap or mild detergent and large amounts of water until no evidence of chemical remains (at least 15-20 minutes). Get medical attention, if needed.

EYE CONTACT: Wash eyes immediately with large amounts of water or normal saline, occasionally lifting upper and lower lids, until no evidence of chemical remains. Get medical attention immediately.

INGESTION: If vomiting occurs, keep head lower than hips to help prevent aspiration. Get medical attention.

 SECTION 5 FIRE FIGHTING MEASURES

FIRE AND EXPLOSION HAZARDS: Slight fire hazard. Dust/air mixtures may ignite or explode. Minimum Ignition Energy: greater than 500 mJ.

EXTINGUISHING MEDIA: regular dry chemical, carbon dioxide, water, regular foam
 Large fires: Use regular foam or flood with fine water spray.

FIRE FIGHTING: Move container from fire area if it can be done without risk. Do not scatter spilled material with high-pressure water streams. Dike for later disposal. Use extinguishing agents appropriate for surrounding fire. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low areas.

 SECTION 6 ACCIDENTAL RELEASE MEASURES

OCCUPATIONAL RELEASE:

Collect spilled material in appropriate container for disposal. Keep out of water supplies and sewers. Keep unnecessary people away, isolate hazard area and deny entry.

SECTION 7 HANDLING AND STORAGE

Store and handle in accordance with all current regulations and standards. Keep separated from incompatible substances.

SECTION 8 EXPOSURE CONTROLS, PERSONAL PROTECTION

EXPOSURE LIMITS:**HATU:**

No occupational exposure limits established.

VENTILATION: Provide local exhaust ventilation system. Ventilation equipment should be explosion-resistant if explosive concentrations of material are present. Ensure compliance with applicable exposure limits.

EYE PROTECTION: Wear splash resistant safety goggles. Provide an emergency eye wash fountain and quick drench shower in the immediate work area.

CLOTHING: Wear appropriate chemical resistant clothing.

GLOVES: Wear appropriate chemical resistant gloves.

RESPIRATOR: Under conditions of frequent use or heavy exposure, respiratory protection may be needed. Respiratory protection is ranked in order from minimum to maximum. Consider warning properties before use.
Any chemical cartridge respirator with organic vapor cartridge(s) and dust and mist filter(s).
Any chemical cartridge respirator with organic vapor cartridge(s) and high-efficiency particulate filter(s).
Any air-purifying respirator with a full facepiece, an organic vapor canister and a dust, mist, and fume filter.
Any powered, air-purifying respirator with a full facepiece and a high-efficiency particulate filter.

For Unknown Concentrations or Immediately Dangerous to Life or Health -
Any supplied-air respirator with full facepiece and operated in a pressure-demand or other positive-pressure mode in combination with a separate escape supply.
Any self-contained breathing apparatus with a full facepiece.

SECTION 9 PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE: solid

COLOR: off-white

PHYSICAL FORM: solid (at 20 degrees C and 101.3 kPa)

ODOR: Not available

MOLECULAR WEIGHT: 380.23

MOLECULAR FORMULA: C10-H15-N6-O.F6-P
 BOILING POINT: Not applicable
 MELTING POINT: 358-361 F (181-183 C) (decomposes at 183-189 degrees C with evolution of gas)
 VAPOR PRESSURE: Not applicable
 VAPOR DENSITY: Not applicable
 SPECIFIC GRAVITY: Not available
 WATER SOLUBILITY: Not available
 PH: Not applicable
 VOLATILITY: Not applicable
 ODOR THRESHOLD: Not available
 EVAPORATION RATE: Not applicable
 COEFFICIENT OF WATER/OIL DISTRIBUTION: Not available

 SECTION 10 STABILITY AND REACTIVITY

REACTIVITY: Stable at normal temperatures and pressure.

CONDITIONS TO AVOID: Avoid heat, flames, sparks and other sources of ignition.
 Avoid contact with incompatible materials.

INCOMPATIBILITIES: metals, oxidizing materials

HAZARDOUS DECOMPOSITION:

Thermal decomposition products: miscellaneous decomposition products

POLYMERIZATION: Will not polymerize.

 SECTION 11 TOXICOLOGICAL INFORMATION

HATU:

TARGET ORGANS: immune system (sensitizer)

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: asthma

ADDITIONAL DATA: Acute Oral LD50: No mortality and limited toxicity were noted in rats following a single oral administration (gavage) of 2000 mg/kg HATU. There were no abnormalities observed at necropsy. Skin Sensitization in Animals: HATU was found to cause skin sensitization in a Magnusson-Kligman Maximisation study conducted in guinea pigs. In vitro Mutagenicity: HATU was not mutagenic to Salmonella typhimurium (Ames test) or Escherichia coli. Signs and Symptoms of Exposure: Skin irritation, swelling, rash, shortness of breath, coughing. Potential Routes of Entry: Skin, eye(s), respiratory.

 SECTION 12 ECOLOGICAL INFORMATION

Not available

 SECTION 13 DISPOSAL CONSIDERATIONS

Dispose in accordance with all applicable regulations.

SECTION 14 TRANSPORT INFORMATION

No classification assigned.

SECTION 15 REGULATORY INFORMATION

U.S. REGULATIONS:

TSCA INVENTORY STATUS: N

TSCA 12(b) EXPORT NOTIFICATION: Not listed.

CERCLA SECTION 103 (40CFR302.4): N

SARA SECTION 302 (40CFR355.30): N

SARA SECTION 304 (40CFR355.40): N

SARA SECTION 313 (40CFR372.65): N

SARA HAZARD CATEGORIES, SARA SECTIONS 311/312 (40CFR370.21):

ACUTE: Y

CHRONIC: Y

FIRE: N

REACTIVE: N

SUDDEN RELEASE: N

OSHA PROCESS SAFETY (29CFR1910.119): N

STATE REGULATIONS:

California Proposition 65: N

EUROPEAN REGULATIONS:

EC NUMBER: Not assigned.

EC RISK AND SAFETY PHRASES:

R 42/43 May cause sensitization by inhalation and skin contact.

S 7 Keep container tightly closed.

S 22 Do not breathe dust.

S 24/25 Avoid contact with skin and eyes.

S 36 Wear suitable protective clothing.

SECTION 16 OTHER INFORMATION

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CONFIDENTIAL

Inveresk Report No. 16386

1-Hydroxy-7-azabenzotriazole (HOAT)
TESTING FOR MUTAGENIC ACTIVITY WITH *Salmonella typhimurium*
TA 1535, TA 1537, TA 98 AND TA 100 AND *Escherichia coli* WP2uvrA

Inveresk Project No. 762358

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Total Number of Pages: 37

AUTHENTICATION

'I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the OECD Principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.'



C G Riach BSc HNC
Study Director

Date: Oct 9.98

QUALITY ASSURANCE STATEMENT

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a predetermined schedule.

This report has been audited by Inveresk Quality Assurance Personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

Inveresk Project No. 762358

Inveresk Report No. 16386

Signed: Guian Farou
(Quality Assurance)

Date: 6 October 1998

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PERSONNEL INVOLVED IN PROJECT 762358

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SUMMARY

HOAT was tested for mutagenic activity in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2uvrA at concentrations ranging from 17 to 5000 μg per plate.

The tests were conducted on agar plates in the presence and absence of an Aroclor 1254 induced rat liver preparation and co-factors (S9 mix) required for mixed-function oxidase activity.

Concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the S9 mix.

The results obtained in both mutation assays were similar. Mutagenic activity was observed in *Salmonella typhimurium* strains TA 1535, TA 98, TA 100 and *Escherichia coli*, in the presence of S9 mix, and in *Salmonella typhimurium* strain TA 98 and *Escherichia coli* in the absence of S9 mix.

No precipitation of the test material was observed.

Toxicity to the bacteria was noted in the absence of S9 mix only at 5000 μg per plate in strains TA 98 and TA 100 in both mutation assays, and in strain TA 1537 in the first mutation assay.

It was concluded that 1-Hydroxy-7-azabenzotriazole was mutagenic to *Salmonella typhimurium* and *Escherichia coli*, when tested in dimethylsulphoxide up to a predetermined maximum limit.

INTRODUCTION

The purpose of this study was to establish the potential of HOAT to induce gene mutation in *Salmonella typhimurium* and *Escherichia coli*. The specific damage caused by a mutagen may not be detectable using a particular strain of bacteria. This is because the DNA site coding for a feature selected in the test system may not be mutable by the type of agent under examination. It is necessary, therefore, to use a variety of bacterial strains in order to test for a broad range of chemical mutagens. At the present time, available data suggest that the use of the 5 strains used in this project permits the detection of a wide spectrum of mutagens.

It is well recognised, however, that many chemicals which may be reactive in a mammalian cell, following metabolic activation, are quite inactive in bacterial cells. Extracts of mammalian cells are combined, therefore, with the bacterial indicator cells in a tissue mediated assay to increase the relevance of the test in assessing the mutagenicity of chemicals to man.

The methods used in this study conform to OECD Guideline No. 471 and 472, the European Commission Annex V Test Method B13 and B14, ICH Guidelines CPMP/ICH/141/95 Step 4 and the USA EPA Pesticide Assessment Guidelines Sub-division F, Series 84-2.

This report describes the methods used and the results obtained in tests carried out at the Elphinstone Research Centre laboratories of Inveresk Research, Tranent.

Key dates in the conduct of the study:

Study Initiation:	8 June 1998
Experimental Start Date:	3 July 1998
Experimental Completion Date:	17 July 1998

Study Completion Date:

Please see Authentication page for date of Study Director's signature (Final reports only).

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research for 5 years after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the disposal or continued storage of raw data.

EXPERIMENTAL PROCEDURE

The procedures used are based on the method of Ames *et al* (1975). Aseptic techniques conducted under amber light were used throughout.

MATERIALS

Test Substance

1-Hydroxy-7-azabenzotriazole (HOAT), Batch No. 216696, a light brown powder, was received from Perkin Elmer on 10 June 1998 and was stored refrigerated in the dark.

Positive Controls

Positive control substances used were 2-aminoanthracene (2-AAN), sodium azide (NaN_3), 9-aminoacridine (9-AA), 2-nitrofluorene (2-NF) and N-ethyl-N-nitro-N-nitrosoguanidine (ENNG).

The positive control substances, except sodium azide, were dissolved in dimethylsulphoxide. Sodium azide was dissolved in sterile, ultra-pure water.

Test Solution

Dimethylsulphoxide (DMSO) was used to dissolve and dilute HOAT.

Vehicle Control

Dimethylsulphoxide (DMSO) was used as the vehicle control.

Inducer

The inducer was the polychlorinated biphenyl mixture, Aroclor 1254.

Animals

The animals used to prepare the activation system were male Fischer 344 rats.

Agar Plates

The Vogel-Bonner Medium E agar plates with 2% glucose used in the Ames test were prepared in-house using purified agar.

Bacteria

Salmonella typhimurium

Four strains of *Salmonella typhimurium* were used:

S. typhimurium TA 1535

S. typhimurium TA 1537

S. typhimurium TA 98

S. typhimurium TA 100

They were obtained in 1976 from Professor B N Ames, Department of Biochemistry, University of California, Berkeley, CA, USA, and stored in liquid nitrogen since that time until used.

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 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 mutation which appears to have added a $\begin{matrix} \text{G} \\ \text{---} \\ \text{C} \end{matrix}$ base-pair resulting in $\begin{matrix} \text{GGGG} \\ \text{CCCC} \end{matrix}$. 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 $\begin{matrix} \text{CGCGCG} \\ \text{GCGCGC} \end{matrix}$ which is reverted with the deletion of 2 base-pairs, $\begin{matrix} \text{CG} \\ \text{---} \\ \text{GC} \end{matrix}$. It is readily reverted by aromatic amines and derivatives.

All 4 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.

Strains TA 100 and TA 98 carry a plasmid which the other *S. typhimurium* strains do not. The plasmid, R-Utrecht (pKM101), 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 (pKM101) carries resistance to ampicillin. It is not yet clear why the presence of this particular R-factor should increase the sensitivity of these strains to the mutagenicity of certain chemicals. The involvement of an error-prone repair mechanism has been postulated.

Escherichia coli

One strain of *Escherichia coli* was used. This was *E. coli* WP2*uvrA*. The strain was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland in 1976. This strain contains an ochre mutation in the *trpE* locus and can be mutated to tryptophan independence either by a base-pair reversion of an A-T base-pair in the *trpE* locus, or more likely, by a base-pair substitution within a number of transfer RNA loci elsewhere in the chromosome. The latter causes the original defect to be suppressed (ochre suppression) and involves only base pair substitution transitions at G-C base pairs.

Thus, while the *trp*⁺ reversion system can detect mutations resulting from chemical attack at both A-T and G-C base pairs, it does not detect frameshift mutagens. The *uvrA* mutation causes the bacteria to be deficient in the excision of bulky lesions from the DNA, so, it is more readily mutated by certain agents (ultraviolet radiation, polycyclic hydrocarbons).

METHODS

Preparation of the Metabolic Activation System

Animals

Male Fischer 344 rats (average weight 244 g) were injected once intraperitoneally with Aroclor 1254 (diluted in corn oil to a concentration of 200 mg.ml⁻¹) at a dosage of 500 mg.kg⁻¹. They were allowed drinking water continuously, but food was withheld for 16 h before they were killed in an atmosphere of carbon dioxide 5 days after injection.

Preparation of 9,000 g Supernatant Fluid (S9 Mix) from Livers

Freshly killed animals were totally immersed in cold 2% Tego (an ampholytic detergent), then excess fluid was wiped off. The abdomen was opened and the liver removed, taking special care not to cut the gastro-intestinal tract. Livers from several animals were collected in a tared beaker containing ice-cold 0.15 M KCl.

The beaker was weighed and the 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 and homogenised by 8 strokes of a glass tube vessel while the Teflon pestle (radial clearance 0.14-0.15 mm) rotated at about 1,200 r.p.m. The homogenate was transferred to sterile polypropylene centrifuge tubes and spun at a relative centrifugal force of 9,000 g for 10 min at 0° to +2°C. The supernatant fluid was decanted leaving behind a thick pellet of (mainly) whole cells, nuclei and mitochondria.

Post-mitochondrial supernatant fluid was prepared in sufficient quantity for the experiment and stored, as 2 ml and 5 ml samples in sterile plastic tubes, immersed in liquid nitrogen (-196°C).

Enzyme Properties of the 9,000 g Supernatant Fluid

Details of the batch of S9 mix used in the mutation experiments are shown in Appendix 3 together with responses from positive control pre-mutagens in the Ames test using *S. typhimurium* strain TA 1538.

Preparation of S9 Mix

Ice-cold 0.05 M phosphate buffer, pH 7.4, was added to the following pre-weighed reagents to give final concentrations in S9 mix of:

NADP di-Na salt	4 mM (= 3.150 mg.ml ⁻¹)
Glucose-6-phosphate di-Na salt	25 mM (= 7.605 mg.ml ⁻¹)
MgCl ₂ .6H ₂ O	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 S9 mix.

Preparation of Bacteria

Samples of each strain were grown by culturing for 16 h at 37°C in nutrient broth (25 g Oxoid Nutrient Broth No. 2.litre⁻¹). These cultures were kept for up to 2 days at +4°C to allow relevant checks to be performed but fresh cultures were used for the experiments.

Preparation of the Assay Plates

Diluted agar (0.6% Difco Bacto-agar, 0.6% NaCl) was sterilised by autoclaving. L-histidine and biotin solutions, and L-tryptophan solutions were sterilised by filtration.

For use with *S. typhimurium* strains, 5 ml of sterile 1.0 mM L-histidine.HCl, 1.0 mM biotin solution was added to each 100 ml of soft agar, and for *E. coli* WP2uvrA, 1.0 ml of 1.35 mM L-tryptophan was added to each 100 ml agar. Each of the agars (with additions) were thoroughly mixed prior to use and maintained in a water bath at 45°C.

The tests were performed using the Direct Plate method.

The Direct Plate Method

2 ml volumes of soft agar were dispensed into small sterile tubes. To this 0.5 ml of S9 mix or 0.05 M phosphate buffer, pH 7.4 were added followed by 0.1 ml of bacteria. 100 µl of solvent or test solution was added last.

The tube contents, which were continually cooling, were mixed and poured onto minimal medium prepared in-house. These plates contained 25 ml of 1.5% purified agar in Vogel-Bonner Medium E (Vogel *et al* (1956)) with 2% glucose. When the soft agar had set, the plates were inverted and incubated at 37°C for 2 days.

At the end of the incubation period the colonies were counted using a Biotran III automated counter (New Brunswick Incorporated, NJ, USA) at maximum sensitivity ie colonies of 0.1 mm or more in diameter counted. The plates were also examined for precipitates and, microscopically, for microcolony growth.

Toxicity Test

A toxicity test using strain TA 100 only was performed in the presence and absence of S9 mix to establish suitable dose levels for the mutation tests. One plate of each of the following concentrations of HOAT was used:

17, 50, 167, 500, 1667 and 5000 μg per plate.

Mutation Tests

Two independent mutation tests were conducted using 5 bacterial strains (*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* WP2uvrA). The dose levels for both the mutation assays, based on the results of the toxicity test, were:

17, 50, 167, 500, 1667 and 5000 μg per plate.

Triplicate plates were prepared for each bacterial strain and dose level in both the presence and absence of S9 mix.

Quality Control

At the times that the experiments were conducted, each strain was tested for its resistance to ampicillin (indicating the presence of pKM101) and sensitivity to ultraviolet (u.v.) light and crystal violet (indicating persistence of the *uvrB* and *rfa*

mutations). In addition, the following control groups were established, triplicate plates being poured for each mean datum point.

1. Dimethylsulphoxide (DMSO), 100 μ l, used as the test compound vehicle, in both the presence and the absence of S9 mix.
2. With S9 Mix
2-Aminoanthracene (2-AAN), 20 μ g per plate with *E. coli*, 2 μ g per plate with TA 1535 and TA 1537 and 0.5 μ g per plate with TA 98 and TA 100, used to demonstrate activity of the S9 mix and the mutability of the bacteria.
3. Without S9 Mix
N-ethyl-*N*-nitro-*N*-nitrosoguanidine (ENNG), 2 μ g per plate with *E. coli*; Sodium azide (NaN_3), 1 μ g per plate, with TA 1535 and TA 100; 2-Nitrofluorene (2-NF), 1 μ g per plate, with TA 98; 9-aminoacridine (9-AA), 80 μ g per plate, with TA 1537. These substances served as an aid to strain identification and to demonstrate the mutability of the bacteria.

Evaluation of Results

A test was considered acceptable if for each strain:

- i) the bacteria demonstrated their typical responses to crystal violet, ampicillin and u.v. light.

- ii) at least 2 of the vehicle control plates were within the following ranges: TA 1535, 4-30; TA 1537, 1-20; TA 98, 10-60; TA 100, 60-200 and *E. coli* WP2uvrA 1-60.
- iii) on at least 2 of the positive control plates there were $\times 2$ the mean vehicle control mutant numbers per plate, or in the case of TA 100, $\times 1.5$ the mean vehicle control mutant numbers per plate.
- If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required on at least 2 of the positive control plates.
- iv) no toxicity or contamination was observed in at least 4 dose levels.
- v) in cases where a mutagenic response was observed, that no more than one dose level was discarded before the dose which gave the highest significant mean colony number.

Where these criteria were met, a significant mutagenic response was recorded if there was:

- i) for *S. typhimurium* strains TA 1535, TA 1537 and TA 98 and for *E. coli*, at least a doubling of the mean concurrent vehicle control values at some concentration of the test substances and, for *S. typhimurium* strain TA 100, a 1.5-fold increase over the control value. If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required before a significant mutagenic response was identified.

- ii) a dose related response, although at high dose levels this relationship could be inverted because of, for example, (1) toxicity to the bacteria generally, (2) specific toxicity to the mutants and (3) inhibition of foreign compound metabolising enzymes where mutagens require metabolic activation by the liver.

- iii) a reproducible effect in independent tests.

RESULTS AND DISCUSSION

Toxicity Test

The results of the toxicity test on HOAT are shown in Table 1.

No toxicity to the bacteria was observed. There was no precipitation of the test material.

Mutation Tests

The average numbers of *his*⁺ and *trp*⁺ revertant colonies per dose level obtained in the main tests are shown in Tables 2-5 whilst the individual plate counts are displayed in Appendices 1-2.

Quality Control

The strains of *S. typhimurium* and *E. Coli* were sensitive to crystal violet, whereas only the plasmid-containing strains, TA 98 and TA 100, were resistant to ampicillin. The strains were also tested for sensitivity to u.v. light emitted over a period of 5-10 s from a lamp set at 254 nm. Increased sensitivity to u.v. light was demonstrated. These results are consistent with the known properties of these bacteria.

Vehicle Control Groups

The vehicle control values were generally within the normal ranges experienced in this laboratory and reported in the literature with these strains of *S. typhimurium* and *E. coli*, (Ames *et al.*, 1975; Gatehouse *et al.*, 1994).

Positive Control Groups

The results obtained in the positive control groups were within the normal ranges expected for each bacterial strain and activation condition.

Test Rejection

All tests were acceptable according to the study criteria.

HOAT

In both mutation assays, 1-Hydroxy-7-azabenzotriazole induced reproducible mutagenic responses in both the presence and absence of S9 mix.

In the first mutation assay, in the presence of S9 mix, a dose-related mutagenic response was observed with strain TA 98. Strains TA 1535, TA 100 and E coli, also met the criteria stated for a mutagenic response at the highest concentration of 5000 μg per plate. In the absence of S9 mix, significant dose-related mutagenic responses were also obtained with strain TA 98 and E coli.

Toxicity to the bacteria was apparent, in the absence of S9 mix with strains TA 1537, TA 98 and TA 100. Slightly thin background lawns of microcolonies were observed at 5000 μg per plate.

The results of the second mutation assay were broadly similar to the first assay. In the presence of S9 mix, dose related mutagenic responses were obtained in strains TA 98 and *Escherichia coll.* Significant mutagenic responses were also noted in strains TA 1535 and TA 100 at the highest concentration of 5000 μg per plate. In the absence of S9 mix, mutagenic responses were observed again at 5000 μg per plate, with TA 98 and *Escherichia coli*. The results noted met the criteria stated for a mutagenic response.

Toxicity was apparent in the form of slightly thin background lawns of microcolonies, at the highest concentration with strains TA 98 and TA 100.

The response in the presence of S9 mix in both assays, was stronger with *Salmonella typhimurium* strain TA 98 than with TA 1535, TA 100 or *Escherichia coli*.

No precipitation of the test material was observed.

CONCLUSION

It was concluded that 1-Hydroxy-7-azabenzotriazole was mutagenic to *Salmonella typhimurium* and *Escherichia coli*, when tested in dimethylsulphoxide up to a predetermined maximum limit.

REFERENCES

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Vogel H J and Bonner D M (1956). Acetylornithinase of *E. coli*: partial purification and some properties. *J Biol Chem.* 218, 97-106.

TABLE 1

Test 1

HOAT - revertant colony numbers obtained per plate using
bacterial strain :- TA 100

Strain	Dose level μ g/plate	Liver S-9	Individual revertant colony counts
TA 100	Solvent	-	121
	17	-	102
	50	-	75
	167	-	98
	500	-	106
	1667	-	102
	5000	-	60
	Solvent	+	68
	17	+	81
	50	+	73
	167	+	82
	500	+	75
	1667	+	70
	5000	+	77

- : Absence
+ : Presence

TABLE 2

Test 2
 Mean Number of *his+* and *trp+* Revertant Colonies Obtained when 4 Strains of *S. typhimurium* and one Strain of *E. coli* were Treated with HOAT in the Presence of a Post-mitochondrial Fraction (S9 Mix) from the Livers of Male Rats Treated with Aroclor 1254 (FLI 087)

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± S.D.				
DMSO	100 μl	8 ± 1	5 ± 2	14 ± 4	98 ± 10	8 ± 5
HOAT	17	6 ± 3	4 ± 3	20 ± 3	105 ± 12	10 ± 5
	50	12 ± 2	6 ± 3	25 ± 3	99 ± 12	9 ± 2
	167	8 ± 2	6 ± 2	43 ± 3	109 ± 5	9 ± 3
	500	14 ± 2	6 ± 3	101 ± 10	116 ± 6	11 ± 2
	1667	17 ± 7	9 ± 5	210 ± 16	134 ± 3	18 ± 2
	5000	35 ± 8	6 ± 1	199 ± 5	150 ± 3	50 ± 7
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 μg	2 μg	0.5 μg	0.5 μg	20 μg
	Mean ± S.D.	244 ± 7	175 ± 27	260 ± 62	445 ± 28	353 ± 19

S.D. Standard Deviation

2AAN 2-Aminoanthracene

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 3

Test 2
 Mean Number of *his+* and *trp+* Revertant Colonies Obtained when 4 Strains of *S. typhimurium* and one Strain of *E. coli* were Treated with HOAT in the Absence of S9 Mix

Substance	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
DMSO	100 µl	7 ± 2	5 ± 3	16 ± 2	81 ± 9	9 ± 2
HOAT	17	5 ± 1	3 ± 1	11 ± 2	90 ± 3	10 ± 2
	50	8 ± 3	5 ± 2	12 ± 1	89 ± 5	11 ± 4
	167	7 ± 2	3 ± 2	13 ± 2	90 ± 13	9 ± 1
	500	5 ± 2	6 ± 3	21 ± 2	88 ± 9	11 ± 4
	1667	8 ± 3	4 ± 1	26 ± 4	73 ± 3	21 ± 4
	5000	7 ± 1	7 ± 4 (STL)	55 ± 3 (STL)	71 ± 8 (STL)	35 ± 6
Positive Controls	Compound	NaN ₃	9AA	2NF	NaN ₃	ENNG
	Dose Level	1 µg	80 µg	1 µg	1 µg	2 µg
	Mean ± S.D.	145 ± 21	475 ± 97	165 ± 20	251 ± 8	186 ± 13

S.D. Standard Deviation

NaN₃ Sodium azide
 2NF 2-Nitrofluorene

9AA 9-Aminoacridine
 ENNG N-Ethyl-N-nitro-N-nitrosoguanidine

STL : SLIGHTLY THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 4

Test 3
 Mean Number of *his+* and *trp+* Revertant Colonies Obtained when 4 Strains of *S. typhimurium* and one Strain of *E. coli* were Treated with HQAT in the Presence of a Post-mitochondrial Fraction (S9 Mix) from the Livers of Male Rats Treated with Aroclor 1254 (FL1 087)

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
DMSO	100 μl	9 \pm 2	3 \pm 1	12 \pm 1	99 \pm 3	5 \pm 3
HQAT	17	6 \pm 2	4 \pm 1	17 \pm 4	100 \pm 4	8 \pm 2
	50	9 \pm 3	7 \pm 1	25 \pm 3	106 \pm 5	9 \pm 4
	167	6 \pm 3	3 \pm 3	43 \pm 12	91 \pm 13	9 \pm 3
	500	10 \pm 5	7 \pm 2	83 \pm 6	101 \pm 12	11 \pm 2
	1667	18 \pm 3	6 \pm 4	195 \pm 38	131 \pm 3	20 \pm 3
	5000	38 \pm 2	11 \pm 3	234 \pm 74	151 \pm 1	43 \pm 18
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 μg	2 μg	0.5 μg	0.5 μg	20 μg
	Mean \pm S.D.	182 \pm 8	213 \pm 4	144 \pm 20	317 \pm 44	401 \pm 38

S.D. Standard Deviation

2AAN 2-Aminoanthracene

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 5

Test 3
 Mean Number of *his*⁺ and *trp*⁺ Revertant Colonies Obtained when 4 Strains of
S. typhimurium and one Strain of *E. coli* were Treated with HOAT
 in the Absence of S9 Mix

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
DMSO	100 μl	5 \pm 2	2 \pm 1	12 \pm 3	69 \pm 7	4 \pm 2
HOAT	17	7 \pm 3	3 \pm 2	11 \pm 5	92 \pm 10	4 \pm 2
	50	12 \pm 4	1 \pm 1	7 \pm 2	78 \pm 7	3 \pm 2
	167	6 \pm 2	2 \pm 1	11 \pm 2	78 \pm 5	6 \pm 0
	500	10 \pm 6	2 \pm 2	11 \pm 3	64 \pm 3	5 \pm 4
	1667	11 \pm 4	3 \pm 2	19 \pm 6	84 \pm 7	15 \pm 4
	5000	13 \pm 4	3 \pm 2	27 \pm 4 (STL)	70 \pm 4 (STL)	43 \pm 11
Positive Controls	Compound	NaN ₃	9AA	2NF	NaN ₃	ENNG
	Dose Level	1 μg	80 μg	1 μg	1 μg	2 μg
	Mean \pm S.D.	131 \pm 12	474 \pm 47	166 \pm 29	221 \pm 21	190 \pm 22

S.D. Standard Deviation

NaN₃ Sodium azide

2NF 2-Nitrofluorene

9AA 9-Aminoacridine

ENNG N-Ethyl-N-nitro-N-nitrosoguanidine

STL : SLIGHTLY THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

APPENDIX 1

Test 2

HOAT - revertant colony numbers obtained per plate using bacterial strains :- TA 1535, TA 1537, TA 98, TA 100 and WP2uvrA

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	Solvent	-	7	2	8, 9, 5
	17	-	5	1	5, 6, 5
	50	-	8	3	11, 8, 5
	167	-	7	2	5, 8, 9
	500	-	5	2	7, 3, 4
	1667	-	8	3	7, 5, 11
	5000	-	7	1	7, 6, 7
	Solvent	+	8	1	7, 8, 8
	17	+	6	3	8, 8, 3
	50	+	12	2	14, 12, 10
	167	+	8	2	7, 8, 10
	500	+	14	2	12, 13, 16
	1667	+	17	7	9, 23, 20
	5000	+	35	8	32, 44, 29
	TA 1537	Solvent	-	5	3
17		-	3	1	2, 2, 4
50		-	5	2	7, 4, 3
167		-	3	2	2, 2, 6
500		-	6	3	9, 5, 4
1667		-	4	1	3, 5, 5
5000		-	7	4	4 STL, 5 STL, 12 STL
Solvent		+	5	2	5, 6, 3
17		+	4	3	7, 4, 2
50		+	6	3	3, 9, 6
167		+	6	2	8, 6, 4
500		+	6	3	2, 8, 7
1667		+	9	5	4, 10, 13
5000		+	6	1	5, 7, 5
TA 98		Solvent	-	16	2
	17	-	11	2	12, 13, 9
	50	-	12	1	11, 12, 12
	167	-	13	2	12, 13, 15
	500	-	21	2	22, 19, 22
	1667	-	26	4	21, 29, 28
	5000	-	55	3	54 STL, 58 STL, 53 STL
	Solvent	+	14	4	14, 18, 10
	17	+	20	3	17, 22, 20
	50	+	25	3	25, 27, 22
	167	+	43	3	46, 40, 44
	500	+	101	10	104, 90, 109
	1667	+	210	16	191, 217, 221
	5000	+	199	5	201, 203, 193

SD : Standard Deviation

- : Absence
+ : Presence

STL : SLIGHTLY THIN LAWN TL : THIN LAWN

Continued on next page.

APPENDIX 1 (continued)

Test 2

HOAT - revertant colony numbers obtained per plate using bacterial strains :- TA 1535, TA 1537, TA 98, TA 100 and WP2uvrA

Strain	Dose level $\mu\text{g}/\text{plate}$	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	Solvent	-	81	9	91, 76, 76
	17	-	90	3	93, 87, 89
	50	-	89	5	92, 84, 92
	167	-	90	13	105, 83, 82
	500	-	88	9	78, 91, 96
	1667	-	73	3	70, 75, 73
	5000	-	71	8	67 STL, 67 STL, 80 STL
	Solvent	+	98	10	95, 89, 109
	17	+	105	12	119, 99, 98
	50	+	99	12	85, 105, 107
	167	+	109	5	114, 108, 105
	500	+	116	6	120, 110, 119
	1667	+	134	3	137, 132, 132
	5000	+	150	3	149, 148, 153
	WP2uvrA	Solvent	-	9	2
17		-	10	2	8, 12, 9
50		-	11	4	8, 15, 9
167		-	9	1	8, 9, 9
500		-	11	4	6, 12, 14
1667		-	21	4	16, 24, 23
5000		-	35	6	39, 38, 29
Solvent		+	8	5	3, 12, 10
17		+	10	5	15, 5, 10
50		+	9	2	9, 7, 10
167		+	9	3	12, 7, 9
500		+	11	2	9, 13, 11
1667		+	18	2	16, 20, 18
5000		+	50	7	44, 57, 50

SD : Standard Deviation

- : Absence

+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

APPENDIX 1 (continued)

Test 2

Mutability test with bacterial strains :-
TA 1535, TA 1537, TA 98, TA 100 and WP2uvrA

Strain	Compound	Dose level per plate		Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	NaN3	1	µg	-	145	21	169, 133, 134
TA 1537	9AA	80	µg	-	475	97	363, 521, 540
TA 98	2NF	1	µg	-	165	20	144, 168, 183
TA 100	NaN3	1	µg	-	251	8	247, 246, 260
WP2uvrA	ENNG	2	µg	-	186	13	196, 171, 191
TA 1535	2AAN	2	µg	+	244	7	248, 247, 236
TA 1537	2AAN	2	µg	+	175	27	149, 173, 203
TA 98	2AAN	0.5	µg	+	260	62	190, 279, 310
TA 100	2AAN	0.5	µg	+	445	28	457, 465, 413
WP2uvrA	2AAN	20	µg	+	353	19	347, 374, 338

SD : Standard Deviation

- : Absence

+ : Presence

NaN3 Sodium azide
2NF 2-Nitrofluorene
2AAN 2-Aminoanthracene

9AA 9-Aminoacridine
ENNG N-Ethyl-N-nitro-N-nitrosoguanidine

APPENDIX 2

Test 3

HOAT - revertant colony numbers obtained per plate using bacterial strains :- TA 1535, TA 1537, TA 98, TA 100 and WP2uvrA

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	Solvent	-	5	2	7, 4, 4
	17	-	7	3	4, 10, 6
	50	-	12	4	17, 9, 10
	167	-	6	2	6, 4, 8
	500	-	10	6	16, 8, 5
	1667	-	11	4	15, 8, 9
	5000	-	13	4	11, 10, 17
	Solvent	+	9	2	8, 12, 8
	17	+	6	2	7, 4, 8
	50	+	9	3	6, 9, 12
	167	+	6	3	3, 8, 8
	500	+	10	5	6, 10, 15
	1667	+	18	3	16, 17, 22
	5000	+	38	2	38, 40, 36
	TA 1537	Solvent	-	2	1
17		-	3	2	4, 1, 3
50		-	1	1	1, 1, 2
167		-	2	1	3, 1, 2
500		-	2	2	1, 2, 4
1667		-	3	2	2, 5, 2
5000		-	3	2	2, 5, 3
Solvent		+	3	1	2, 4, 4
17		+	4	1	3, 5, 5
50		+	7	1	8, 7, 6
167		+	3	3	6, 1, 2
500		+	7	2	7, 8, 5
1667		+	6	4	3, 10, 5
5000		+	11	3	11, 8, 13
TA 98		Solvent	-	12	3
	17	-	11	5	11, 16, 7
	50	-	7	2	7, 6, 9
	167	-	11	2	12, 9, 13
	500	-	11	3	9, 10, 14
	1667	-	19	6	14, 17, 25
	5000	-	27	4	31 STL, 23 STL, 26 STL
	Solvent	+	12	1	11, 12, 12
	17	+	17	4	16, 21, 14
	50	+	25	3	23, 28, 23
	167	+	43	12	48, 52, 29
	500	+	83	6	79, 80, 90
	1667	+	195	38	206, 152, 226
	5000	+	234	74	170, 217, 315

SD : Standard Deviation

- : Absence
+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

Continued on next page.

APPENDIX 2 (continued)

Test 3

HOAT - revertant colony numbers obtained per plate using bacterial strains :- TA 1535, TA 1537, TA 98, TA 100 and WP2uvrA

Strain	Dose level μ g/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	Solvent	-	69	7	75, 62, 69
	17	-	92	10	100, 94, 81
	50	-	78	7	70, 80, 83
	167	-	78	5	77, 83, 73
	500	-	64	3	65, 66, 61
	1667	-	84	7	77, 84, 90
	5000	-	70	4	72 STL, 73 STL, 66 STL
	Solvent	+	99	3	99, 101, 96
	17	+	100	4	103, 102, 96
	50	+	106	5	101, 110, 107
	167	+	91	13	91, 78, 103
	500	+	101	12	100, 89, 113
	1667	+	131	3	134, 129, 130
	5000	+	151	1	150, 151, 151
WP2uvrA	Solvent	-	4	2	5, 5, 1
	17	-	4	2	6, 2, 4
	50	-	3	2	2, 5, 1
	167	-	6	0	6, 6, 6
	500	-	5	4	6, 1, 9
	1667	-	15	4	19, 12, 15
	5000	-	43	11	36, 38, 56
	Solvent	+	5	3	4, 8, 3
	17	+	8	2	7, 8, 10
	50	+	9	4	6, 8, 14
	167	+	9	3	7, 7, 13
	500	+	11	2	13, 12, 9
	1667	+	20	3	18, 18, 23
	5000	+	43	18	63, 29, 37

SD : Standard Deviation

- : Absence

+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

APPENDIX 2 (continued)

Test 3

Mutability test with bacterial strains :-
TA 1535, TA 1537, TA 98, TA 100 and WP2uvrA

Strain	Compound	Dose level per plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	NaN3	1 μ g	-	131	12	132, 142, 119
TA 1537	9AA	80 μ g	-	474	47	429, 471, 522
TA 98	2NF	1 μ g	-	166	29	199, 154, 146
TA 100	NaN3	1 μ g	-	221	21	225, 240, 198
WP2uvrA	ENNG	2 μ g	-	190	22	168, 191, 212
TA 1535	2AAN	2 μ g	+	182	8	185, 173, 188
TA 1537	2AAN	2 μ g	+	213	4	212, 217, 209
TA 98	2AAN	0.5 μ g	+	144	20	167, 131, 133
TA 100	2AAN	0.5 μ g	+	317	44	349, 334, 267
WP2uvrA	2AAN	20 μ g	+	401	38	375, 383, 444

SD : Standard Deviation

- : Absence

+ : Presence

NaN3 Sodium azide
2NF 2-Nitrofluorene
2AAN 2-Aminoanthracene

9AA 9-Aminoacridine
ENNG N-Ethyl-N-nitro-N-nitrosoguanidine

APPENDIX 3

In vitro Activation Preparation Form
Activation Batch: FLI 087

PREPARATION Operator: Wanda Zajac Sex: Male Animal: Rat Strain: Fischer 344 Supplier: Harlan Olac Limited Organ: Liver Total grams of organ: 226 Details of spin: 9,000 g supernatant Total volume of preparation (ml): 678 Storage temperature: -196°C		Date prepared: 9 June 1998 Date of Expiry: 9 December 1998 Number of animals: 20 Average animal weight (g): 244 Date induced: 4 June 1998 Inducer: Aroclor 1254 Supplier: Monsanto (UK) Limited Preparation solution: 0.15 M KCl Number of vials prepared: 97 x 5 ml			
METABOLIC ACTIVATION Operator: Pam Cattanach Strain: <i>S. typhimurium</i> TA 1538 Date plated: 9 June 1998 Culture batch: F088 Batch No. (plates): 000346 Date counted: 11 June 1998					
Substance	Quantity per Plate	Revertant Colonies per Plate			Mean
Dimethylsulphoxide	100 µl	17	10	10	12
2-Aminoanthracene	0.5 µg	612	616	404	544
2-Acetylaminofluorene	10 µg	484	519	374	459
4-Acetylaminofluorene	1 µg	77	68	63	69
Benzo(a)pyrene	5 µg	191	206	179	192
Dimethylaminoazobenzene	100 µg	72	68	64	68
QUALITY ASSURANCE		Auditor: Gillian Birnie Date Audited: 1 July 1998			