

BASF

Abteilung Toxikologie
Department of Toxicology

6700 Ludwigshafen
West Germany

en-fu/66

JAN 19 1987

REPORT

on the Study of

**2,4,6-Triamino-p-
(carbo-2'-ethyl-hexyl-1'-oxi)-
1,3,5-triazene**

(ZNT Test Substance No. 86/200)

in the

AMES TEST

(Standard Plate Test with
Salmonella typhimurium)

(Project No.: 40/1M0200/86)

Testing facility: BASF Aktiengesellschaft
Department of Toxicology Z 470
6700 Ludwigshafen/Rh., FRG

This report consists of 12 pages and 8 tables.

Dieses Dokument enthält Betriebs- und Geschäftsgeheimnisse der BASF. Es ist Eigentum der BASF und darf nur zu dem von BASF vorgesehenen Zweck verwendet werden. Jede andere oder darüber hinausgehende Verwendung, Verwertung, Weitergabe, Vervielfältigung oder Veröffentlichung bedarf der Einwilligung der BASF.

This document contains manufacturing and trade secrets of BASF. It is the property of BASF and may be used only for that purpose for which it was intended by BASF. Every other or additional use, exploitation, reproduction, publication or submission to other parties require the written permission of BASF.

CONTENTS

	Page
1. SUMMARY	1
2. INTRODUCTION	2
3. MATERIAL AND METHOD	3
3.1. Test substance	3
3.2. Tissue preparation	4
3.2.1. S-9 fraction	4
3.2.2. S-9 mix	5
3.3. Bacteria	5
3.4. Mutagenicity tests	7
3.5. Titer determination	8
3.6. Checking out the tester strains	8
3.7. Controls	9
3.7.1. Negative control	9
3.7.2. Positive controls	9
3.8. Evaluation criteria	10
3.9. Tester strains, doses, number of plates	10
3.10. Retention of records	10
4. RESULTS	11
4.1. Mutagenicity tests	11
4.1.1. Tests without S-9 mix	11
4.1.2. Tests with S-9 mix	11
4.2. Toxicity	11
4.3. Solubility	11
5. LITERATURE	12
Annex: Tables 1 to 8	
STATEMENT of the quality assurance unit	

Project No.: 40/1M0200/86

1. SUMMARY

The substance 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'oxi)-1,3,5-triazene was tested for mutagenicity in the Ames test.

Strains: TA 1535, TA 100, TA 1537, TA 98

Dose range: 20 µg - 5000 µg/plate

Test conditions: Standard plate test without and with metabolic activation (S-9 mix).

Solubility: Incomplete solubility of the test substance in DMSO from about 500 µg/plate onward.

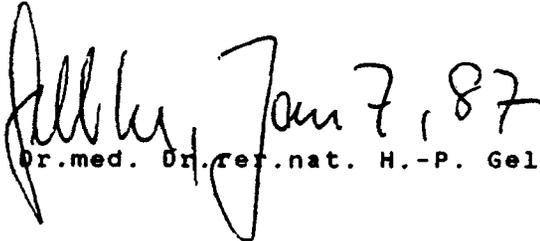
Toxicity: No bacteriotoxic effect (reduced his⁻ background growth) was observed.

Mutagenicity:

An increase in the number of his⁺ revertants was not observed either without S-9 mix or after the addition of a metabolizing system.

Assessment:

According to the results of the present study, the test substance is not mutagenic in the Ames test under the experimental conditions chosen here.


Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke


Dr.rer.nat. G. Engelhardt
(Study director)

Project No.: 40/1M0200/86

2. **INTRODUCTION**

The Ames test is a short-term test in bacteria (1, 2) and is used as a screening method for detecting a gene mutagenic effect of chemical substances.

Since most of the substances are not mutagenic or carcinogenic themselves, but only after metabolic transformation, and since the main part of all metabolic processes is catalyzed by the enzyme systems of the liver, the Ames test is carried out not only directly, but also in the presence of a metabolizing system obtained from rat livers. For this purpose, rats are pretreated with Aroclor 1254 for an activation of the enzymes which metabolize foreign substances.

Project No.: 40/1M0200/86

3. MATERIAL AND METHOD**3.1. Test substance**

Name of test substance: 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'oxi)-1,3,5-triazene

Batch No.: 18301/142

Test substance No.: 86/200

Degree of purity: > 95 %

Appearance,
consistency: White powder

Solvent: DMSO

Storage: +4°C

The stability of the test substance throughout the study period will be verified analytically by reanalysis at a later date. The results of this analysis may be requested from the sponsor.

The stability of the test substance in the solvent DMSO was determined analytically.

More detailed information about the test substance can be found in the raw data and may be requested from the sponsor (ME/Z; BASF Aktiengesellschaft).

Project No.: 40/1M0200/86

3.2. Tissue preparation

3.2.1. S-9 fraction

The S-9 fraction is prepared according to Ames et al. (2).

5 male Sprague-Dawley rats (200 - 300 g) receive a single intraperitoneal injection of 500 mg Aroclor 1254 (as a 20% solution in peanut oil - w/v) per kg body weight 5 days before sacrifice.

During this time the animals are housed in Makrolon cages in air-conditioned rooms. The day/night rhythm is 12 hours (light period from 6.00 - 18.00 hours and dark period from 18.00 - 6.00 hours).

Standardized pelleted feed and tap water from bottles are available ad libitum.

5 days after administration the rats are sacrificed, and the livers are prepared (all preparation steps for obtaining the liver microsome enzymes are carried out using sterile solvents and glassware at a temperature of +4°C). The livers are weighed and washed in an equivalent volume of a 150 mM KCl solution (1 ml $\hat{=}$ 1 g wet liver), then cut into small pieces and homogenized in three volumes of KCl solution. After centrifugation of the homogenate at 9000 x g for 10 minutes at +4°C, 5-ml portions of the supernatant (so-called S-9 fraction) are quickly deep-frozen in dry ice and stored at -70°C to -80°C for 2 months at the most.

Preparation of S-9 fraction: June 30, 1986
(1st and 2nd experiments).

Project No.: 40/1M0200/86

3.2.2. S-9 mix

The S-9 mix is prepared freshly prior to each experiment (1, 2). For this purpose, a sufficient amount of S-9 fraction is thawed at room temperature and 3 volumes of S-9 fraction are mixed with 7 volumes of S-9 supplement (cofactors). This preparation, the so-called S-9 mix, is kept on ice until used. The concentrations of the cofactors in the S-9 mix are:

MgCl ₂	8 mM
KCl	33 mM
glucose-6-phosphate	5 mM
NADP	4 mM
phosphate buffer (pH 7.4)	100 mM.

The phosphate buffer is prepared by mixing an Na₂HPO₄ solution (25.42 g/l) with an NaH₂PO₄ solution (22.28 g/l) in a ratio of about 4 : 1.

3.3. Bacteria

The rate of induced back mutations of several bacteria mutants from histidine auxotrophy to histidine prototrophy is determined (2, 3, 4). The indicator organisms TA 1535, TA 1537, TA 98 and TA 100 selected by Ames especially for this purpose are derivatives of *Salmonella typhimurium*. All strains have a defective excision repair system (uvrB), which prevents the repair of lesions which are induced in the DNA, and this deficiency results in greatly enhanced sensitivity of some mutagens. Furthermore, all strains show a considerably reduced hydrophilic polysaccharide layer (rfa), which leads to an increase in permeability to lipophilic substances.

Project No.: 40/1M0200/86

The strains TA 1535 and TA 100 are derived from histidine-prototrophic Salmonella strains by the substitution mutation his G 46 and are used to detect base pair substitutions. TA 1537 and TA 98 are strains for the detection of frameshift mutagens. These strains carry different frameshift markers, i.e. the +1 mutant his C 3076 in the case of TA 1537 and the +2 type his D 3052 in the case of TA 98.

The strains TA 98 and TA 100 carry an R factor plasmid pKM 101 (4) and, in addition to having genes resistant to antibiotics, they have a modified postreplication DNA repair system, which increases the mutation rate by inducing a defective repair in the DNA; this again leads to a considerable increase in sensitivity.

For testing, deep-frozen (-70°C to -80°C) bacterial cultures (1 ml in 15-ml glass tubes) are thawed at room temperature. 0.1 ml of this bacterial suspension is inoculated in nutrient broth solution (8 g Difco bacto nutrient broth + 5 g NaCl/liter) and incubated in the shaking water bath at 37°C for 16 hours. As a rule, a germ density of $\geq 10^8$ bacteria/ml is reached. These cultures grown overnight are kept in iced water from the beginning of the experiment until the end in order to prevent further growth.

Project No.: 40/1M0200/86

3.4. Mutagenicity tests

The experimental procedure is based on the method of Ames et al. (1, 2).

Test tubes containing 2 ml portions of soft agar which consists of 100 ml agar (0.6% agar + 0.6% NaCl) and 10 ml amino acid solution (minimal amino acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin) are kept in a water bath at 45°C, and the remaining components are added in the following order:

0.1 ml test solution
0.1 ml bacterial suspension
0.5 ml S-9 mix (in tests with metabolic activation)
or
0.5 ml phosphate buffer (in tests without metabolic activation)

After mixing, the samples are poured onto the Vogel-Bonner agar plates (minimal glucose agar plates) within approx. 30 seconds.

Composition of the minimal glucose agar:

980 ml aqua dest.
20 ml Vogel-Bonner E medium
15 g Difco bacto agar
20 g D-glucose, monohydrate.

After incubation at 37°C for 48 hours in the dark, the bacterial colonies (his^+ revertants) are counted.

Project No.: 40/1M0200/86

3.5. Titer determination

In general, the titer is determined only in the experiments with S-9 mix both without test substance (solvent only) and after adding the two highest doses of test substance. For this purpose, 0.1 ml of the overnight cultures (see 3.3.) is diluted to 10^{-6} in each case. Test tubes containing 2 ml portions of soft agar containing maximal amino acid solution for titer determination (5 mM histidine + 0.5 mM biotin) are kept in a water bath at 45°C, and the remaining components are added in the following order:

0.1 ml solvent (without and with test substance)
0.1 ml bacterial suspension (dilution: 10^{-6})
0.5 ml S-9 mix

After mixing, the samples are poured onto the Vogel-Bonner agar plates within approx. 30 seconds. After incubation at 37°C for 48 hours in the dark, the bacterial colonies are counted.

3.6. Checking out the tester strains

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (Δ uvrB); ampicillin resistance (R factor plasmid).

Histidine auxotrophy is automatically checked in each experiment via the spontaneous rate.

Project No.: 40/1M0200/86

3.7. Controls**3.7.1. Negative control**

Parallel with each experiment with and without S-9 mix, a negative control (solvent control, sterility control) is carried out for each tester strain in order to determine the spontaneous mutation rate.

3.7.2. Positive controls

The following positive control substances are used to check the mutability of the bacteria and the activity of the S-9 mix:

with S-9 mix	10 µg 2-aminoanthracene (dissolved in DMSO) for the strains TA 100, TA 98, TA 1537 and TA 1535
without S-9 mix	5 µg N-methyl-N'-nitro-N- nitroso-guanidine (MNNG) (dissolved in DMSO) for the strains TA 100 and TA 1535
	10 µg 4-nitro-o-phenylenediamine (dissolved in DMSO) for the strain TA 98
	100 µg 9-aminoacridine chloride monohydrate (dissolved in DMSO) for the strain TA 1537.

Project No.: 40/1M0200/86

3.8. Evaluation criteria

In general, a substance to be characterized as positive in the Ames test has to fulfill the following requirements:

- doubling of the spontaneous mutation rate (control)
- dose-response relationship
- reproducibility of the results.

3.9. Tester strains, doses, number of plates**1st Experiment**

Strains: TA 1535, TA 100, TA 1537, TA 98
Doses: 0, 20, 100, 500, 2500 and 5000 µg/plate
Solvent: DMSO
Type of test, standard plate test with and without
test condition: S-9 mix
Number of 3 test plates per dose or per control
plates:

2nd Experiment

Strains: TA 1535, TA 100, TA 1537, TA 98
Doses: 0, 20, 100, 500, 2500 and 5000 µg/plate
Solvent: DMSO
Type of test, standard plate test with and without
test condition: S-9 mix
Number of 3 test plates per dose or per control
plates:

3.10. Retention of records

The raw data, protocol and the original of this report will be stored at BASF Aktiengesellschaft at least for the period of time specified in the GLP regulations.

Project No.: 40/1M0200/86

4. RESULTS (Tables 1 - 8)

The substance 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'oxi)-1,3,5-triazene was tested for mutagenicity in the Ames test (standard plate test) both in the presence and in the absence of a metabolizing system obtained from rat liver (S-9 mix) using the strains TA 1535, TA 100, TA 1537 and TA 98.

4.1. Mutagenicity tests**4.1.1. Tests without S-9 mix**

TA 1535:

TA 100: No increase in the number of

TA 1537: his⁺ revertants

TA 98:

4.1.2. Tests with S-9 mix

TA 1535:

TA 100: No increase in the number of

TA 1537: his⁺ revertants

TA 98:

4.2. Toxicity

No bacteriotoxic effect (reduced his⁻ background growth) was observed.

4.3. Solubility

Incomplete solubility of the test substance in DMSO from about 500 µg/plate onward.

Project No.: 40/1M0200/86

5. **LITERATURE**

1. Ames, B.N.; Durston, W.W.; Yamasaki, E.; Lee, F.D.:

Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection.

Proc. Nat. Acad. Sci. USA, 70, 2281 - 2285 (1973)

2. Ames, B.N.; McCann, J.; Yamasaki, E.:

Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test.

Mut. Res., 31, 347 - 364 (1975)

3. Ames, B.N.; Lee, F.D.; Durston, W.E.:

An improved bacterial test system for the detection and classification of mutagens and carcinogens.

Proc. Nat. Sci. USA, 70, 782 - 786 (1973)

4. McCann, J.; Spingarn, N.E.; Kobori, J.; Ames B.N.:
Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids.

Proc. Nat. Acad. Sci. USA, 72, 979 - 983 (1975)

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 1

STUDY NUMBER: 86/79/
STUDY DIREC. : ENI
OPERATOR : SCHI
DATE : 11.07.8/

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA1535

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL.	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	22 17 14	18	4	22 24 21	22	2	36 44 31	1.0	1.0
20	17 21 13	17	4	15 19 18	17	2		1.0	0.8
100	12 17 17	15	3	22 19 20	20	2		0.9	0.9
500~	15 19 15	16	2	26 17 13	19	7		0.9	0.8
2500~	15 17 15	16	1	16 15 18	16	2	27 30 33	0.9	0.7
5000~	22 22 13	19	5	15 17 21	18	3	23 30 23	1.1	0.8
POSITIVE CONTROL 2-AA 10				247 226 178	217	35			9.7
POSITIVE CONTROL MNNG 5	1130 1780 1350	1420	331					80.4	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
~: PRECIPITATION

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 2

STUDY NUMBER: 86/79/
STUDY DIREC. : ENI
OPERATOR : SCHI
DATE : 11.07.86

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA 100

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL.	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	115 111 127	118	8	106 109 108	108	2	20 15 19	1.0	1.0
20	114 105 111	110	5	105 105 104	105	1		0.9	1.0
100	90 107 104	100	9	108 113 95	105	9		0.9	1.0
500~	100 115 116	110	9	107 105 92	101	8		0.9	0.9
2500~	101 112 86	100	13	114 102 112	109	6	15 16 15	0.8	1.0
5000~	110 124 124	119	8	118 112 102	111	8	14 25 19	1.0	1.0
POSITIVE CONTROL 2-AA 10				2100 2000 2150	2083	76			19.3
POSITIVE CONTROL MNNG 5	2000 1800 1890	1897	100					16.1	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
~: PRECIPITATION

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 3

STUDY NUMBER: 86/79/
STUDY DIREC.: ENI
OPERATOR : SCHI
DATE : 11.07.86

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA1537

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	13 11 11	12	1	12 11 9	11	2	13 12 10	1.0	1.0
20	13 7 9	10	3	14 13 11	13	2		0.8	1.2
100	11 8 7	9	2	9 12 10	10	2		0.7	1.0
500~	8 9 7	8	1	9 7 8	8	1		0.7	0.8
2500~	8 10 10	9	1	6 6 3	5	2	12 16 12	0.8	0.5
5000~	11 7 5	8	3	4 2 8	5	3	13 12 15	0.7	0.4
POSITIVE CONTROL 2-AA 10				159 211 195	188	27			17.7
POSITIVE CONTROL AAC 100	1220 1090 958	1089	131					93.4	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
~: PRECIPITATION

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 4

STUDY NUMBER: 86/79/
STUDY DIREC.: ENI
OPERATOR : SCHI
DATE : 11.07.8

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA98

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL.	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	21 23 25	23	2	31 33 31	32	1	39 27 41	1.0	1.0
20	21 22 32	25	6	38 41 30	36	6		1.1	1.1
100	25 27 27	26	1	35 35 30	33	3		1.1	1.1
500~	22 23 26	24	2	32 31 36	33	3		1.0	1.0
2500~	26 21 23	23	3	40 31 31	34	5	34 21 23	1.0	1.1
5000~	20 23 21	21	2	26 28 34	29	4	17 11 7	0.9	0.9
POSITIVE CONTROL Z-AA 10				1050 1040 1480	1190	251			37.6
POSITIVE CONTROL NPD 10	789 872 821	827	42					36.0	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
~: PRECIPITATION

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 5

STUDY NUMBER: 86/79/2
STUDY DIREC. : ENC
OPERATOR : SCHW
DATE : 18.07.86

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA1535

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL.	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	16 13 13	14	2	15 16 19	17	2	33 36 34	1.0	1.0
20	13 11 14	13	2	16 20 13	16	4		0.9	1.0
100	14 13 12	13	1	12 10 13	12	2		0.9	0.7
500~	11 12 15	13	2	15 16 17	16	1		0.9	1.0
2500~	14 10 13	12	2	13 20 15	16	4	40 35 37	0.9	1.0
5000~	14 13 15	14	1	19 18 19	19	1	23 17 19	1.0	1.1
POSITIVE CONTROL 2-AA 10				203 176 230	203	27			12.2
POSITIVE CONTROL MNNG 5	1470 1830 1430	1577	220					112.6	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
~: PRECIPITATION

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 6

STUDY NUMBER: 86/79/2
STUDY DIREC. : ENC
OPERATOR : SCHW
DATE : 18.07.86

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA100

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL.	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	110 116 113	113	3	104 105 112	107	4	11 25 14	1.0	1.0
20	97 104 100	100	4	105 114 105	108	5		0.9	1.0
100	108 109 109	109	1	115 95 110	107	10		1.0	1.0
500~	106 110 82	99	15	126 97 107	110	15		0.9	1.0
2500~	- 108 68	88	28	112 108 98	106	7	17 21 20	0.8	1.0
5000~	94 90 91	92	2	104 96 94	98	5	14 10 13	0.8	0.9
POSITIVE CONTROL 2-AA 10				1630 1620 1590	1613	21			15.1
POSITIVE CONTROL MNNG 5	1300 1350 1470	1373	87					12.2	

* : S-9 FRACTION/COFACTORS = 3:7
- : CONTAMINATION ~: PRECIPITATION

EXP : EXP. TO 10

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 7

STUDY NUMBER: 86/79/1
STUDY DIREC. : ENI
OPERATOR : SCHI
DATE : 18.07.86

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA1537

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL.	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE CONTROL DMSO	9 - 10	10	1	8 10 11	10	2	20 22 16	1.0	1.0
20	7 9 6	7	2	10 11 11	11	1		0.8	1.1
100	8 10 12	10	2	13 8 13	11	3		1.1	1.2
500~	9 6 8	8	2	11 12 14	12	2		0.8	1.3
2500~	7 5 11	8	3	8 5 4	6	2	15 18 8	0.8	0.6
5000~	8 5 6	6	2	5 4 11	7	4	13 13 14	0.7	0.7
POSITIVE CONTROL 2-AA 10				119 130 122	124	6			12.8
POSITIVE CONTROL AAC 100	471 375 769	538	205					56.7	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
-: CONTAMINATION ~: PRECIPITATION

B. A. S. F. A. G.
DEPARTMENT OF TOXICOLOGY

TABLE : 8

STUDY NUMBER: 86/79/1
STUDY DIREC. : ENC
OPERATOR : SCH
DATE : 18.07.84

AMES TEST WITH : 86/200
METHOD : STANDARD PLATE TEST

STRAIN: TA98

DOSE MCG/PL	REVERTANTS / PLATE						TITER DIL	QUOTIENT	
	-S9	M	SD	+S9*	M	SD	EXP-6	-S9	+S9*
NEGATIVE	22	23	1	31	31	1	56	1.0	1.0
CONTROL	22			31			60		
DMSO	24			32			56		
20	22	22	1	34	32	2		1.0	1.0
	23			30					
	22			31					
100	23	22	3	29	31	2		1.0	1.0
	24			30					
	19			33					
500~	20	20	3	29	32	3		0.9	1.0
	23			32					
	18			34					
2500~	19	20	1	32	32	1	42	0.9	1.0
	20			33			40		
	20			31			37		
5000~	16	19	3	16	18	3	38	0.8	0.6
	19			21			37		
	21			18			40		
POSITIVE CONTROL 2-AA 10				1610 1590 1360	1520	139			48.5
POSITIVE CONTROL NPD 10	900 934 906	913	18					40.3	

* : S-9 FRACTION/COFACTORS = 3:7 EXP : EXP. TO 10
~: PRECIPITATION

STATEMENT

of the quality assurance unit

Number of test substance: 86/200

Name of test substance: 2,4,6-Trianiilino-p-(carbo-2' -ethyl-hexyl-1' -oxi)-
1,3,5-triazene

Type of study: Ames-Test (Standard Plate Test with Salmonella
typhimurium)

The quality assurance unit inspected the study, audited the final report, and reported findings to the study director and to management.

Date of inspection	Report to study director and to management
July 3, 1986	July 11, 1986
July 11, 1986	July 11, 1986
Dez. 18, 1986	Dez. 23, 1986

Ludwigshafen/Rhein, Jan. 15, 1987


.....
Signature QAU