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MAR 06 1987

Cytogenetic Investigations in  
NMRI Mice  
After a Single Oral Administration  
of

2,4,6-Triamino-1,3,5-triazine  
-1,3,5-triazine

Micronucleus Test  
(Project No. 26M0200/8618)

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This report consists of 19 pages and 8 tables.

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STATEMENT of the quality assurance unit

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1. **SUMMARY**

The substance 2,4,6-trianilino-p-(carbo-2-ethyl-hexyl-1-oxi)-1,3,5-triazine was tested for mutagenicity in NMRI mice using the micronucleus test method. For this purpose, 2,4,6-trianilino-p-(carbo-2-ethyl-hexyl-1-oxi)-1,3,5-triazine, suspended in olive oil, was administered once orally to male and female animals at dose levels of 2100 mg/kg, 1050 mg/kg and 525 mg/kg body weight in a volume of 10 ml/kg body weight in each case.

For control purposes, male and female mice were administered merely the olive oil by the same route.

As a positive control, 40 mg of cyclophosphamide/kg body weight, dissolved in aqua dest., was administered once orally to male and female animals in a volume of 10 ml/kg body weight.

Animals which were administered the olive oil or the positive control substance cyclophosphamide did not show any clinical signs of toxicity.

The doses of 2100 mg/kg, 1050 mg/kg and 525 mg/kg body weight led to irregular respiration and piloerection about 15 minutes after test substance administration. The signs of toxicity were observed for about 4 - 5 hours in the intermediate and low dose groups; in the highest dose group of 2100 mg/kg body weight on the day after treatment these signs were no longer found.

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The animals were sacrificed and the bone marrow of the two femora was prepared 16, 24 and 48 hours after administration in the highest dose group of 2100 mg/kg body weight. In the test groups of 1050 mg/kg and 525 mg/kg body weight in the negative control group and in the positive control group the 24-hour sacrifice intervals were investigated only. After staining of the preparations 1000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 1000 polychromatic erythrocytes were also registered.

According to the results of the present study, the single oral administration of 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine in doses of 2100 mg/kg, 1050 mg/kg and 525 mg/kg body weight did not lead to an increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was always in the same range as that of the control in all dose groups and at all sacrifice intervals.

No inhibition of erythropoiesis determined from the ratio of polychromatic to normochromatic erythrocytes was detected.

Thus, under the experimental conditions chosen here, the test substance 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine does not have any chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis.

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## 2. INTRODUCTION

The aim of the present study was to test the substance 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine for mutagenicity in NMRI mice after a single oral administration using the micronucleus test method. The test procedure and the preparation of the bone marrow were based on the method of SCHMID, W. (10,11) and SALAMONE, M. et al. (9).

The micronucleus test (1,2,3,4,8,9,10,11,12) is a method independent of the karyotype of the test animal (1,8) for an indirect detection of a chromosome-damaging (clastogenic) effect or a damage of the mitotic apparatus (spindle poison effect) (6,7,9,10,11,12).

Micronuclei (Howell-Jolly bodies) are scored primarily in young, polychromatic erythrocytes which contain one or sometimes more than one micronucleus occurring as a round or seldom as a crescent-shaped or bizarre structure. The size of micronuclei may indicate the mode of action of the test substance. Thus, chromosome-breaking agents primarily induce small micronuclei which are formed from acentric chromosome fragments (6,7,10,11,12) and the diameter of which is about 1/20 to 1/5 (< 1/4) of the cell diameter. Substances which induce impairments of distribution in the course of mitosis, however, lead to an increase in large micronuclei which have a diameter  $\geq$  1/4 of the cell diameter and which may present entire chromosomes (6,10,12).

The study was carried out in September 1986 in accordance with the OECD guideline for testing of chemicals - "Genetic Toxicology: Micronucleus Test", No. 474.

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### 3. MATERIAL AND METHODS

#### 3.1. Test substance

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

Batch No.: 18301/142

Test substance No.: 86/200

Appearance, consistency: White powder

Degree of purity: 98%

Storage: +4°C

Solvent: Olive oil

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 homogeneity of the test substance was guaranteed on account of the high purity.

The stability of the test substance in the carrier olive oil 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).

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3.2. Animals

The investigations were carried out in male and female NMRI mice, Charles River GmbH, WIGA, D-8741 Sulzfeld, FRG.

3.3. Housing and diet

For the duration of about one week the animals were housed in Makrolon cages, type M III, in groups of 5 separately according to sex in fully air-conditioned rooms in which central air conditioning guaranteed a range of 20 - 24°C for temperature and a range of 30 - 70% for relative humidity. Before the beginning of the experiment the animals were transferred to Makrolon cages, type M I, and housed individually under the same conditions until the end of the test.

The animals were identified using cage cards.

The day/night rhythm was 12 hours (12 hours light from 6.00 - 18.00 hours and 12 hours darkness from 18.00 - 6.00 hours).

Standardized pelleted feed (Kliba Haltungsdiät, Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland) and drinking water from bottles were available ad libitum.

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3.4. Test groups and doses

Male and female animals were assigned to the test groups according to a randomization plan compiled in the Department of Toxicology of BASF Aktiengesellschaft. The dose levels were fixed as follows:

In the determination of the acute oral toxicity all animals survived the dose of 2150 mg/kg body weight which led to clinical signs of toxicity such as irregular respiration, piloerection and, in some cases, squatting posture. Higher doses suspended in olive oil led to a viscous mass which could no longer be administered.

Therefore, 2100 mg/kg body weight was selected as the highest dose in the present cytogenetic investigations. 1050 mg/kg or 525 mg/kg body weight were administered as further doses.

The substance to be administered per kg body weight was suspended in olive oil.

- Test group II was given 2100 mg test substance/kg body weight or 10 ml/kg body weight of a suspension with a concentration of 21 g/100 ml.
- Test group III was given 1050 mg test substance/kg body weight or 10 ml/kg body weight of a suspension with a concentration of 10.5 g/100 ml.
- Test group IV was given 525 mg test substance/kg body weight or 10 ml/kg body weight of a suspension with a concentration of 5.25 g/100 ml.

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Test groups, the number of animals, and dose levels can be seen from the table below.

Test groups		Sacrifice intervals (hours)	Dose ml or mg/kg b.w.	Number of animals ♂♂ / ♀♀
I	1	24	negative control 10 ml olive oil	5/5*
	2	16	2100 mg test substance 10 ml test substance preparation	5/5
II	3	24	2100 mg test substance 10 ml test substance preparation	5/5
	4	48	2100 mg test substance 10 ml test substance preparation	5/5
III	5	24	1050 mg test substance 10 ml test substance preparation	5/5
IV	6	24	525 mg test substance 10 ml test substance preparation	5/5
V	7	24	40 mg cyclophosphamide 10 ml test solution	5/5

\* The control animals were sacrificed 24 hours after administration of the solvent. The control animals were treated at different times so that it was always possible to prepare control animals simultaneously for all the different sacrifice intervals of the test animals.

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3.5. Analysis

Test substance preparation analysis:

The analytical investigations on the determination of the concentration in the carrier were carried out by means of HPLC. For this purpose, 2 samples per concentration were removed from the test substance preparations using a stomach tube and transferred to test tubes.

The analytical investigations were carried out in the analytical laboratory (ZHU, Dr. Schäfer-Lüderssen).

Feed analysis:

The feed used in the study was assayed for contaminants. In view of the aim and duration of the study the contaminants occurring in commercial feed ought not to influence the results.

Water analysis:

The drinking water is regularly assayed for contaminants by the municipal authorities of Frankenthal and by the Department of Water Chemistry and the Technical Services of BASF Aktiengesellschaft.

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3.6. Test procedure and administration

Animals with a mean weight of 27.9 g were used for the study. Male and female animals per sacrifice interval were given 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine suspended in olive oil at dose levels of 2100 mg/kg, 1050 mg/kg and 525 mg/kg body weight. Treatment consisted of a single oral administration. The volume of administration was 10 ml/kg body weight.

All test substance formulations were prepared immediately before administration.

The amount of substance or volume to be administered was related to the specific weight of the individual animals on the day of the experiment.

For control purposes, male and female animals were given merely the carrier olive oil by the same route.

As a positive control, 40 mg of cyclophosphamide/kg body weight, dissolved in aqua dest., was administered once orally to male and female animals in a volume of 10 ml/kg body weight.

3.7. Clinical examinations

After the administration of the test substance the animals were examined for any evident clinical signs of toxicity.

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### 3.8. Preparation of the bone marrow

The bone marrow was prepared according to the method described by SCHMID, W. (10,11).

- The two femora were prepared from the animals, and all soft tissues were removed.
- After cutting off the epiphyses, the bone marrow was flushed out of the diaphysis into a centrifuge tube in reciprocal directions using a cannula filled with fetal calf serum which was at room temperature (about 2 ml/femur).
- The suspension was mixed thoroughly with a pipette, centrifuged at 1500 rpm for 5 minutes, the supernatant was pipetted off except for a few drops, and the precipitate was resuspended.
- 1 drop of this suspension was dropped onto clean microscopic slides, using a Pasteur pipette. Smears were prepared using slides with ground edges, the preparations were dried in the air and subsequently stained.

#### Staining:

The slides were stained in eosin and methylene blue solution for 5 minutes, rinsed in aqua dest. and then placed in fresh aqua dest. for 2 or 3 minutes. They were finally stained in Giemsa solution for 12 minutes.

After being rinsed twice in aqua dest. and clarified in xylene, the preparations were embedded in Entellan.

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3.9. Microscopic evaluation

As a rule, 1000 polychromatic erythrocytes from each of the male and female animals of every test group are evaluated and investigated for micronuclei. The normochromatic erythrocytes (= normocytes), which occur, are also scored. The following parameters are recorded:

- Number of polychromatic erythrocytes
- Number of polychromatic erythrocytes containing micronuclei

The increase in the micronucleus rate in polychromatic erythrocytes of treated animals as compared with the solvent control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity of the substance tested.

- Number of normochromatic erythrocytes
- Number of normochromatic erythrocytes containing micronuclei

The rate of micronuclei in normochromatic erythrocytes at the early sacrifice intervals represents the situation before test substance administration and may serve as a control value. A substance-induced increase in the number of micronuclei in normocytes may be found with an increase in the duration of the sacrifice intervals.

- Ratio of polychromatic to normochromatic erythrocytes

This ratio indicates an influence of the test substance specifically on the bone marrow.

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- Number of small micronuclei ( $d < D/4$ ) and of large micronuclei ( $d \geq D/4$ ) ( $d$  = diameter of micronucleus,  $D$  = cell diameter)

The size of micronuclei may give an interpretation on the mode of action of the test substance i.e. a clastogenic or a spindle poison effect.

The preparations were coded for analysis.

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**3.10. Statistical evaluation**

Two statistical tests were used to answer the questions of whether there are significant differences between control group and dose groups concerning the rate of micronuclei in polychromatic erythrocytes: first, the exact test according to FISHER, which was applied to register significant differences between the relative frequencies of a characteristic of two groups, and, second, the asymptotic U test according to MANN-WHITNEY (rank test modified according to WILCOXON) (5). The relative frequencies of cells with micronuclei were used as a criterion of the rank determination for the U test. The two tests were calculated at the levels of 95% and 99%. Significances at the 95% level were marked with \* (Fisher Yates Test) and with + (U Test), significances at the 99% level were marked with \*\* (Fisher Yates Test) and with ++ (U Test).

The calculations were carried out in the Computer Center of BASF Aktiengesellschaft (Dipl.-Mathematiker Helmstädter).

**3.11. Retention of records**

The raw data, protocol, reserve sample, and microscopic preparations as well as the original of this report are stored at BASF Aktiengesellschaft at least for the period of time specified in the GLP regulations. The microscopic preparations will be retained only as long as the quality of the material allows an evaluation.

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**4. RESULTS****4.1. Analysis**

The values of the analytical investigations can be seen from the following table.

For the determination of the test substance concentration in the carrier the samples were kept at room temperature until the treatment of the last animal (approximately 1 hour) and then deep-frozen until they were determined analytically.

The homogeneity of the test substance in the carrier was guaranteed by constant stirring during the removal and administration of the test substance formulation.

Test groups	Theoretical values	Values determined analytically
II	210 mg/ml	212 mg/ml
III	105 mg/ml	102 mg/ml
IV	52.5 mg/ml	50 mg/ml

Depending on the dose, about 95 - 101% of the theoretical values could be determined analytically.

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#### 4.2. Clinical examinations

The single oral administration of the carrier in a volume of 10 ml/kg body weight was tolerated by all animals without any signs or symptoms.

About 15 minutes after the test substance administration the doses of 2100 mg/kg, 1050 mg/kg and 525 mg/kg body weight led to irregular respiration and piloerection lasting for about 4 - 5 hours in the intermediate and low dose groups. In the 2100 mg/kg group on the day after treatment these signs were no longer found.

The single oral administration of the positive control substance cyclophosphamide in a dose of 40 mg/kg body weight did not cause any evident signs of toxicity.

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## 4.3. Microscopic evaluation

(Tables 1 to 8)

Table 1:	Summary table: summary of the results of all groups
Table 2:	Results of individual animals of the negative control
Tables 3 - 5:	Results of individual animals of the 2100 mg/kg group
Table 6:	Results of individual animals of the 1050 mg/kg group
Table 7:	Results of individual animals of the 525 mg/kg group
Table 8:	Results of individual animals of the positive control

The single oral administration of the olive oil in a volume of 10 ml/kg body weight led to 1.6% polychromatic erythrocytes containing micronuclei after the 24-hour sacrifice interval.

After the single administration of the highest dose of 2100 mg/kg body weight, 1.1% polychromatic erythrocytes containing micronuclei were found after 16 hours, 1.3% after 24 hours and 1.5% after 48 hours.

In the two lower dose groups rates of micronuclei of about 1.7% (1050 mg/kg group) and 1.5% (525 mg/kg group) were detected after a sacrifice interval of 24 hours in each case.

With 16.5%, however, the positive control substance cyclophosphamide, as expected, led to a clear increase in the rate of polychromatic erythrocytes containing micronuclei at a dose level of 40 mg/kg body weight.

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The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control or in the various dose groups at any of the sacrifice intervals.

Thus the test substance 2,4,6-trinilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine did not lead to any increase in the rate of micronuclei. The number of normochromatic or polychromatic erythrocytes containing small micronuclei ( $d < D/4$ ) or large micronuclei ( $d \geq D/4$ ) did not deviate from the solvent control value at any of the sacrifice intervals.

No inhibition of erythropoiesis induced by the treatment of mice with 2,4,6-trinilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine was detected; the ratio of polychromatic to normochromatic erythrocytes was always in the same range as that of the control values in all dose groups.

#### 4.4. Conclusions

According to the results of the present study, there are thus no biologically relevant, significant differences in the frequency of erythrocytes containing micronuclei either between the solvent control and the 3 dose groups (2100 mg/kg, 1050 mg/kg and 525 mg/kg) or between the various sacrifice intervals (16, 24 and 48 hours). Thus, under the experimental conditions chosen here, the test substance 2,4,6-trinilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine has no chromosome-damaging (clastogenic) effect nor does it lead to any impairment of chromosome distribution in the course of mitosis.

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Table 1

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MICRONUCLEUS TEST

Summary of all test groups

Group	Interval: 16 hours				Interval: 24 hours				Interval: 48 hours			
	Poly- chro- matic eryth- rocytes inves- tigated	Normo- cytes/ 10,000 polychro- matic eryth- rocytes	Cells with micronuclei		Poly- chro- matic eryth- rocytes inves- tigated	Normo- cytes/ 10,000 polychro- matic eryth- rocytes	Cells with micronuclei		Poly- chro- matic eryth- rocytes inves- tigated	Normo- cytes/ 10,000 polychro- matic eryth- rocytes	Cells with micronuclei	
			per 1000 polychro- matic eryth- rocytes	per 1000 normochro- matic eryth- rocytes			per 1000 polychro- matic eryth- rocytes	per 1000 normochro- matic eryth- rocytes			per 1000 polychro- matic eryth- rocytes	per 1000 normochro- matic eryth- rocytes
Solvent control olive oil	-				10.000	2140	1.60	0.93	-			
I Test substance 2100 mg/kg body weight	10.000	2084	1.10	0.48	10.000	2765	1.30	1.08	10.000	2989	1.50	0.33
II Test substance 1050 mg/kg body weight	-				10.000	3268	1.70	1.22	-			
V Test substance 525 mg/kg body weight	-				10.000	3291	1.50	0.61				
Positive control cyclophosphamide 40 mg/kg body weight	-				10.000	4638	16.50 <sup>***</sup>	1.29	-			

Isher-Yates Test                   significance 95% (\*); significance 99% (\*\*)

Test                                   significance 95% (+); significance 99% (++)

Table 2

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MICRONUCLEUS TEST

## Summary

Group I, 1: Solvent control - olive oil  
Sacrifice interval: 24 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN d < 1/4 D	Cells with 1 MN d ≥ 1/4 D	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
1	1000	3	-	-	3	3	134	-
2	1000	1	-	-	1	1	341	-
3	1000	3	-	-	3	3	148	-
4	1000	-	-	-	-	-	135	-
5	1000	3	-	-	3	3	168	1
6	1000	2	-	-	2	2	234	-
7	1000	1	-	-	1	1	273	-
8	1000	1	-	-	1	1	272	-
9	1000	-	-	-	-	-	199	-
10	1000	2	-	-	2	2	236	1
Mean	1000	1.6	-	-	1.6	1.6	214.0	0.2

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 1 - 5 male  
Animal Nos. 6 - 10 female

Table 3

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MICRONUCLEUS TEST

## Summary

Group II, 2: 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine - 2100 mg/kg body weight  
Sacrifice interval: 16 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN $d < 1/4 D$	Cells with 1 MN $d \geq 1/4 D$	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
11	1000	1	-	-	1	1	125	-
12	1000	1	-	-	1	1	152	-
13	1000	3	-	-	3	3	101	-
14	1000	-	-	-	-	-	130	-
15	1000	2	-	-	2	2	166	-
16	1000	-	-	-	-	-	344	-
17	1000	2	-	-	2	2	383	1
18	1000	-	-	-	-	-	197	-
19	1000	1	-	-	1	1	277	-
20	1000	1	-	-	1	1	209	-
Mean	1000	1.1	-	-	1.1	1.1	208.4	0.1

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 11 - 15 male  
Animal Nos. 16 - 20 female

Table 4

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MICRONUCLEUS TEST

## Summary

Group II, 3: 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine - 2100 mg/kg body weight  
Sacrifice interval: 24 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN d < 1/4 D	Cells with 1 MN d ≥ 1/4 D	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
21	1000	3	-	-	3	3	143	-
22	1000	-	-	-	-	-	153	-
23	1000	3	-	-	3	3	186	-
24	1000	-	-	-	-	-	261	-
25	1000	1	-	-	1	1	266	1
26	1000	2	-	-	2	2	321	-
27	1000	2	-	-	2	2	222	-
28	1000	1	-	-	1	1	421	1
29	1000	1	-	-	1	1	408	1
30	1000	-	-	-	-	-	384	-
Mean	1000	1.3	-	-	1.3	1.3	276.5	0.3

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 21 - 25 male  
Animal Nos. 26 - 30 female

Table 5

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## Summary

Group II, 4: 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine - 2100 mg/kg body weight  
Sacrifice interval: 48 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN d < 1/4 D	Cells with 1 MN d ≥ 1/4 D	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
31	1000	-	-	-	-	-	221	-
32	1000	2	-	-	2	2	265	-
33	1000	1	-	-	1	1	214	-
34	1000	2	-	-	2	2	318	-
35	1000	1	-	-	1	1	175	-
36	1000	-	-	-	-	-	352	-
37	1000	2	-	-	2	2	499	-
38	1000	2	-	-	2	2	267	1
39	1000	2	-	-	2	2	389	-
40	1000	3	-	-	3	3	289	-
Mean	1000	1.5	-	-	1.5	1.5	298.9	0.1

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 31 - 35 male  
Animal Nos. 36 - 40 female

Table 6

Project No.: 26M0200/8618  
MICRONUCLEUS TEST

## Summary

Group III, 5: 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine - 1050 mg/kg body weight  
Sacrifice interval: 24 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN $d < 1/4 D$	Cells with 1 MN $d \geq 1/4 D$	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
41	1000	1	-	1	2	2	232	-
42	1000	3	-	-	3	3	373	-
43	1000	2	-	-	2	2	217	-
44	1000	2	-	-	2	2	225	-
45	1000	2	-	-	2	2	352	1
46	1000	-	-	-	-	-	339	-
47	1000	-	-	-	-	-	258	-
48	1000	2	-	-	2	2	436	1
49	1000	1	-	-	1	1	535	1
50	1000	3	-	-	3	3	301	1
Mean	1000	1.6	-	0.1	1.7	1.7	326.8	0.4

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 41 - 45 male  
Animal Nos. 46 - 50 female

Table 7

Project No.: 26M0200/8618  
MICRONUCLEUS TEST

## Summary

Group IV, 6: 2,4,6-trianilino-p-(carbo-2'-ethyl-hexyl-1'-oxi)-1,3,5-triazine - 525 mg/kg body weight  
Sacrifice interval: 24 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN d < 1/4 D	Cells with 1 MN d ≥ 1/4 D	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
51	1000	2	-	-	2	2	224	-
52	1000	3	-	-	3	3	221	-
53	1000	1	-	-	1	1	295	1
54	1000	1	-	-	1	1	284	1
55	1000	1	-	-	1	1	252	-
56	1000	-	1	-	1	1	480	-
57	1000	2	-	-	2	2	435	-
58	1000	2	-	-	2	2	446	-
59	1000	2	-	-	2	2	319	-
60	1000	-	-	-	-	-	335	-
Mean	1000	1.4	0.1	-	1.5	1.5	329.1	0.2

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 51 - 55 male  
Animal Nos. 56 - 60 female

Project No.: 26M0200/8618  
MICRONUCLEUS TEST

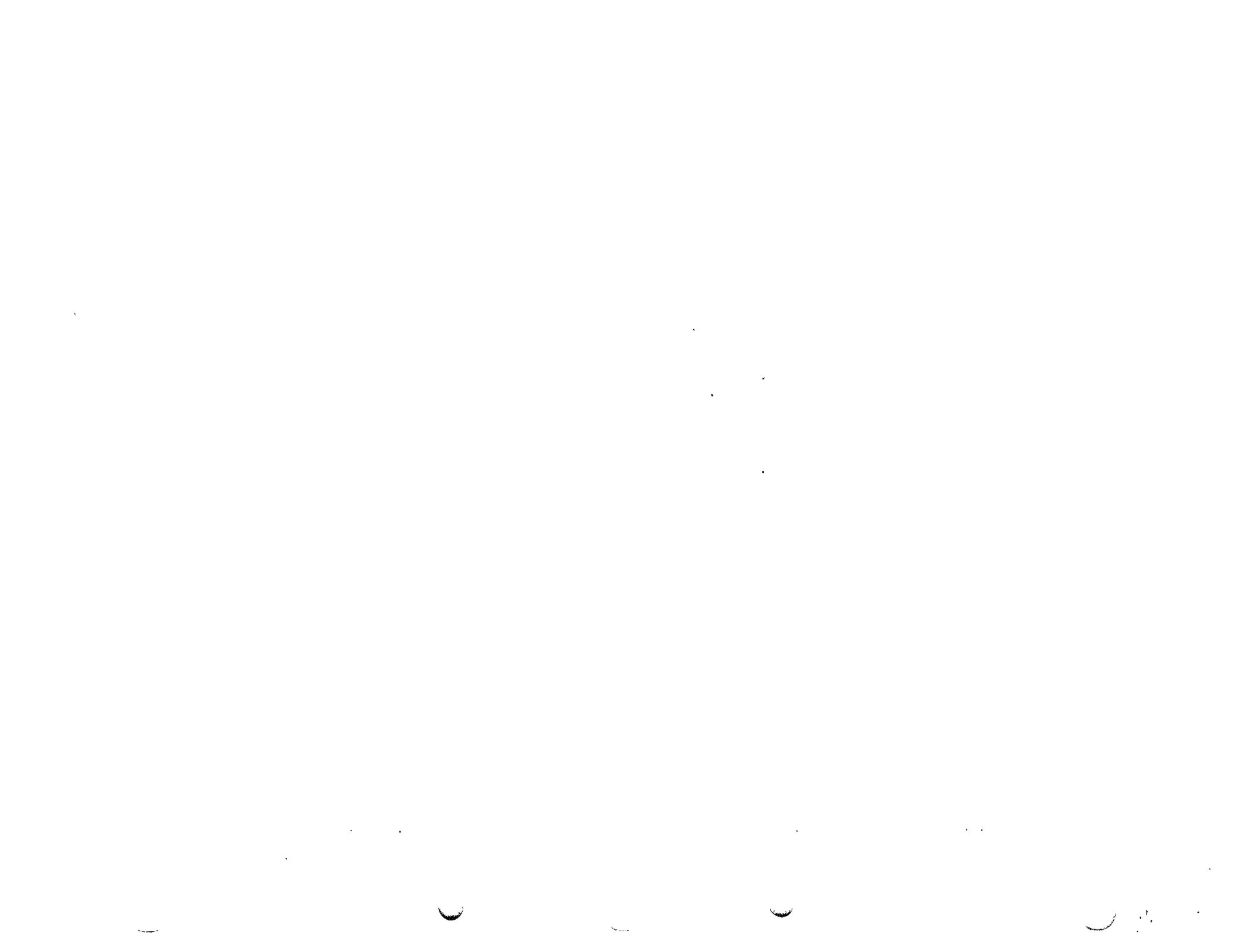
## Summary

Group V, 7: Positive control, cyclophosphamide - 40 mg/kg body weight  
Sacrifice interval: 24 hours

Animal No.	Polychromatic erythrocytes					Normochromatic erythrocytes		
	Total number of cells	Cells with 1 MN $d < 1/4 D$	Cells with 1 MN $d \geq 1/4 D$	Cells with 2 and more MN	Total MN		Total number of cells	Cells with MN
					n	%		
61	1000	15	1	-	16	16	415	1
62	1000	14	-	-	14	14	338	1
63	1000	14	-	-	14	14	318	-
64	1000	18	-	-	18	18	563	1
65	1000	20	-	1	21	21	358	-
66	1000	17	-	-	17	17	629	3
67	1000	10	-	-	10	10	575	-
68	1000	14	-	-	14	14	568	-
69	1000	18	-	-	18	18	526	-
70	1000	22	-	1	23	23	348	-
Mean	1000	16.2	0.1	0.2	16.5	16.5	463.8	0.6

MN = micronucleus  
D = diameter of the cell  
d = diameter of the micronucleus

Animal Nos. 61 - 65 male  
Animal Nos. 66 - 70 female



STATEMENT

of the quality assurance unit

Number of test substance: 86/200

Name of test substance: 2,4,6-Triamylino-p-(Carbo-2'-ethyl-hexyl-1'-oxi) -1,3,5-triazine

Type of study: Cytogenetic Investigations in NMRI Mice After a Single Oral Administration (Micronucleus Test)

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
Sept. 01, 1986	Sept. 12, 1986
Sept. 10, 1986	Sept. 12, 1986
Febr. 26, 1987	Febr. 27, 1987

Ludwigshafen/Rhein, March 6, 1987

*[Handwritten Signature]*  
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Signature QA

