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Maleic hydrazide : an oral carcinogenicity study in rats.

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SUMMARY AND CONCLUSION

Maleic hydrazide is under discussion by different national and international organizations because of contradicting results of a number of carcinogenicity studies carried out in the past. Because maleic hydrazide is used in agriculture rather extensively and is of great importance in the Netherlands as sprouting inhibitor on onions, an oral carcinogenicity study in rats was carried out with 0, 1.0 and 2.0% maleic hydrazide which contained less than 1.5 mg of hydrazine as impurity per kg product.

In this carcinogenicity study as well as in an experiment with mice performed at the International Agency for Research on Cancer (IARC) in Lyon, no treatment related tumours were induced. From these experiments it can be concluded that maleic hydrazide itself is not a carcinogen.

Most likely the contradictory results obtained with maleic hydrazide as described in the literature, can be explained by the presence of relatively high levels of hydrazine as an impurity in the maleic hydrazide that was used in the experiments.

Furthermore the results of this study revealed that 1 and 2% maleic hydrazide in the diet caused proteinuria and increased protein/creatinin ratios in the urine both in males and in females. This points to an effect of maleic hydrazide in the kidneys. This is in good agreement with the observation that degenerative kidney lesions were found with 4% maleic hydrazide in a range-finding experiment of two weeks. However in the longterm study with 1 and 2% of maleic hydrazide no histopathological lesions in the kidneys were observed.

From the study described in this report it can be concluded that on the basis of the effect on the kidneys the no toxic effect level is lower than 1% maleic hydrazide in the diet of rats.

INTRODUCTION

Maleic hydrazide is used extensively as a sprouting inhibitor on onions and potatoes (Noodén 1970). The sprouting inhibitor is effective for several months (Pepino et al 1974) which is due to inhibition of cell division

(Kihlman 1966; Noodén 1969). Maleic hydrazide is very stable and does not decompose during household cooking and frying.

For the evaluation of the acceptability of maleic hydrazide residues on treated crops several toxicity studies have been carried out.

In a two-weeks range-finding study carried out by Kroes et al 1974, 4% of maleic hydrazide induced degenerative kidney lesions while administration of 2% in the diet did not give evidence for kidney damage.

A longterm oral toxicity experiment was carried out in 1955 with the sodium salt of maleic hydrazide. From this study it was concluded that 2% in the diet would be the no-effect level in rats. Moreover with 5% no gross- or histopathological abnormalities were observed (Food Research Laboratories-Inc., unpublished report).

The results of the carcinogenicity studies which are available are contradictory (Barnes et al 1957; Epstein et al 1967; Epstein and Mantel 1968 and Hunter et al 1973). In three studies maleic hydrazide was given by subcutaneous injections to rats or mice. Only in one experiment in mice a dose-related increase in liver cell tumours was found. However, in this study, the maleic hydrazide contained 0,4% of the carcinogen hydrazine as an impurity. From two oral carcinogenicity studies with rats and mice no significant increase in tumour incidence in comparison with the untreated controls were found (Barnes et al 1957, Innes et al 1969).

Because it was believed that the carcinogenic effect found in one experiment could be explained by the presence of hydrazine as impurity, a carcinogenicity study was carried out in which the test substance contained a very low level of this impurity ($\leqslant$ 1.5 mg/kg product).

EXPERIMENT

Material and Methods

Technical maleic hydrazide was obtained in two batches from Aagrunol B.V., Groningen, The Netherlands. The product under investigation, a white powder, contained 10.1% water (w/w). The anhydrous product was stated to be 99% pure in maleic hydrazide as free acid (1,2-dihydro -3,6-pyridazinedione).

Purity of maleic hydrazide was analysed by Ir.J.F.C.Stavenuiter using the method of Yu-Ying Liu et al (1974). Analysis of the hydrazine content at the beginning of the experiment yielded 1.0 and 1.5 mg hydrazine per kg maleic hydrazide for batch 1 and 2 respectively.

Animals

SPF derived Wistar rats from the Institute's own breeding colony were

used. The control and test animals were kept under conventional conditions and were housed in one room (size 4.95 x 2.30 x 2.80). The stainless steel cages (size 32 x 29 x 18 cm) were fitted with wire-mesh floors and fronts. Two animals were kept per cage and all rats had free access to food and drinking water. Room temperature and relative humidity were approximately 22°C and 50% respectively.

Diets

The rats were fed powdered semi-purified stock diet (Muracon SSP-Tox. standard, Trouw N.V. Putten, The Netherlands). The composition of the diet is completely known. The main components are:

casein	18,2%
protein S	4,5%
maizeflour A II	22,4%
glucose	27,3%
cellulose AKU-Floc	10,5%
ketjensil	2,7%
soya oil	4,5%
lard	2,3%
mineralized salts	4,9%
other components	2,7%

The diets were controlled for contaminants and essential elements such as mercury, iodine, lead, cadmium, copper, arsenic, tin, cobalt, aflatoxines B_1, B_2, G_1, G_2, M_1 and chlorinated hydrocarbon pesticides.

For the test groups the maleic hydrazide containing 10.1% water was added to the stock diet at levels of 1.1% and 2.2% (corresponding to 1.0 and 2.0% of maleic hydrazide).

Diets were prepared weekly. For the 1.0% testgroup, 500 g of a premix, containing 110 g maleic hydrazide per kg, was mixed by means of a "Stephan" mixer into 4450 g stock diet. For the 2.0% testgroup 500 g premix containing 220 g maleic hydrazide per kg was mixed into 4450 g stock diet.

The dietary levels of maleic hydrazide were chosen on the basis of the results of a range-finding experiment (Kroes et al, 1974).

Experimental design and conduct

Before the carcinogenicity study a breeding experiment was performed with groups of 50 female and 25 male rats who were fed diets containing 0, 1.0 and 2.0% maleic hydrazide from 1 week before mating onwards. After an one-

week mating period (2 females to 1 male) the dams were separated from the males. The dams were given the respective diets throughout pregnancy and lactating period.

The results of the breeding experiment expressed as fertility index, viability, lactation index and the number of infertile rats was comparable between the groups.

At weaning female and male rats were randomly taken from the various litters from each of the 3 experimental groups and subsequently allocated to 3 groups; one group consisted of each 65 female and 65 male rats (2.0% group) the other two groups consisted of 55 animals of each sex (control and 1.0% group). The carcinogenicity study was conducted with these 3 groups of rats derived and kept on their respective diets for a period of 28 months after weaning. These diets were the same as given to the parent generation.

The study was started 30 september 1974 and the weaned youngs were allocated to their groups and given their respective diets on 4 december 1974. Interim autopsy on 5 male and 5 female rats of each group was performed on 4 december 1975. The study was terminated april 1977.

Behaviour, appearance and mortality

Daily observations were made on general health conditions, appearance, behaviour and mortality of the animals. All observations were recorded and animals which were seriously ill or showed large tumour masses were killed when the condition detoriated.

Growth

The rats were weighed weekly for the first 12 weeks and thereafter every 4 weeks.

Food-intake and water-consumption

Food-intake was recorded at weeks 1, 2, 5, 9 and 12. Water-consumption was measured only at week 18 and 25.

Haematology

The haematological determinations were carried out by Dr.P.W.Helleman of the Unit Clinical Chemistry and Haematology.

Blood samples were collected by orbital punction from 10 male and 10 female rats from each group at 6, 12 and 24 months. The following haemato-

logical parameters were recorded: haemoglobin concentration (Hb), haematocrit value (packed cell volume, PCV), erythrocytes- and total and differential leucocytes count.

Clinical chemistry

From 6 female and 6 male rats of each group blood samples were obtained by orbital punction at 6, 12 and 24 months. The following determinations were carried out: serum alkaline phosphatase (SAP), serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), serum urea and glucose.

Individual urine samples were collected overnight at 3, 6, 12 and 24 months from 8 rats of each sex per group. The following semi-quantitative tests were carried out using Labstix from Ames Laboratories*: pH, protein, occult blood and ketones. After 26 months the osmolality of the urine of 8 female and 8 male rats per group was determinated. In addition urine, protein and creatinin concentrations were measured after 6 and 12 months whereas a phenolsulphtaleine (PSP) clearance test was performed at 12 months with 6-8 female and 5-8 male rats per group.

Organ weights

Absolute organ weights and mean organ/bodyweight ratios of 5 male, and 5 females per group, autopsied after one year were determined. The organs heart, kidneys, liver, spleen, ovaries, testes, brain, thyroid and adrenals were weighed.

Pathology

After 12 months, 5 female and 5 male rats per group were killed by exsanguination during ether anaesthesia followed by a careful search for gross changes. The same procedure was followed with the animals that survived the experimental period of 28 months.

A thorough post-mortem examination was also performed on those animals that died or were killed intercurrently.

From all animals the following tissues were preserved when autolysis was not too advanced; any macroscopic abnormality or lesion suspected of being a tumour that was noticed during gross inspection; in addition all animals of all groups: brain, heart, liver, spleen, kidneys, thyroid, adrenals, ovaries, testes, lungs, pituitary gland, thymus, pancreas, uterus, prostate, mesenteric-

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* The mentioning of specific tradenames is not a recommendation, but is only meant as identification.

and popliteal lymph nodes, submandibular salivary glands, stomach, small- and large intestines, urinary bladder, spinal cord, ischiadic nerve and skeletal muscle.

The tissue samples were fixed in a 4% aqueous, neutral, phosphate buffered, formaldehyde solution. The tissue assigned for histopathological examination were dehydrated and subsequently further processed for paraplastwax (58°C) embedding. The tissue blocks were sliced at 7 µm and stained routinely with haematoxylin-eosin.

Histopathology was carried out at all animals autopsied after 12 months. Histopathological examinations for non-neoplastic changes of the animals that survived the 28 months period were restricted to 10 female and 10 male rats from control- and high dose group.

In order to obtain a clear picture of the incidence, site and type of tumours occuring in the animals during the entire period of the study, a detailed microscopic examination was carried out. All gross tumours and lesions suspected of being a tumour were verified microscopically and the organs taken at autopsy from all surviving and non-surviving female and male rats of the 0, 1.0 and 2.0% treatment groups were searched microscopically for tumours and hyperplastic changes.

Statistics

The experimental data, except pathology, were analysed by the Student's-t-test, and the asterisks indicate: $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$; $^{***}P \leqslant 0.001$.

RESULTS

Behaviour, appearance and mortality

No differences between control and test rats were seen in respect to mortality, general health condition and behaviour. The cumulative mortality in the various groups is given in table 1.

In the female animals of both testgroups a slight increase in mortality was noticed. This increase took place especially in the last 6 months of the experiment.

Occasionally age-related clinical signs developed in all groups towards the end of the study, such as rough yellow coats, ischaemic appearance, dyspnoea, weight-loss and paralysis. These phenomena were as frequent amongst the control as the test groups.

Growth

Initial body-weights and bodyweight gain are given in table 2. A significant reduction in weight gain was noticed in female rats that received maleic hydrazide at both dietary levels during the first half year of the study. The reduced body-weights seem to disappear when the animals reach their adult weight.

Food consumption

Food consumption during the first 12 weeks is summarized in table 3. No clear influence of maleic hydrazide in the diet on food-intake was found.

Water consumption

Table 4 summarizes water consumption during week 18 and 25. There is a marked significant increase in water consumption at the 2.0% dietary level in both female and male rats. In the 1.0% dietary level a tendency to an increased water-intake was present.

Haematology

The results of the haematological examinations are summarized in table 5 and 6. After 6 months there was a significant lower haemoglobine value and haematocrit value in the females of the highest dose group. These effects were transient and not present at 12 and 24 months and were therefore not considered to be important with respect to the toxicity of maleic hydrazide.

Clinical chemistry

The results of the clinical chemical investigations in blood after 6, 12 and 24 months are given in table 7. In the highest dose level the SGOT value was decreased significant in both sexes after 6 months, but not after 12 and 24 months. Urea levels were decreased after 6 months in the 1.0% level males, and in both sexes of the highest dose level. After 24 months no differences were found.

The results of the semi-quantitative determinations in the urine after 3, 6, 12 and 24 months are summarized in table 8, and demonstrate in males a slight increase of urine protein content after 12 and 24 months. After the same periods there are some animals with blood in the urine in both test groups.

Table 9 shows the osmolality values of the urine at 26 months. Urine of the animals of the highest dose level showed a non-significant decrease in osmolality, which means that there is a tendency to a decreased concentration

capacity of the kidneys.

In table 10 protein and creatinin content of the urine at 6 and 12 months and PSP-clearance values after 12 months are given. This table demonstrates significantly increased urine protein values and increased protein/creatinin ratios are evident in both female and male rats at 1.0 and 2.0% maleic hydrazide after 6 and 12 months.

The observed changes in the composition of the urine may be an indication of an effect of maleic hydrazide on the kidneys.

Organ weights

Absolute organ weights and mean organ/body weight ratios from rats killed at 12 months are presented in tables 11 and 12 respectively. No significant changes were noticed that could be related to the administration of maleic hydrazide, except perhaps the increased spleen weight in the highest dose level.

Pathology

The histopathological abnormalities noticed after 12 months are summarized in table 13. The observed changes were equally distributed amongst the various groups and did not suggest a relationship with the treatment.

Macroscopic examination of rats after 12 and 28 months and of the intercurrently autopsied animals showed findings such as inflammatory foci in the lungs, spotted discoloured kidneys, enlargement of pituitary glands and adrenals, pale enlarged livers and evidence of neoplastic growth. These observations were almost equally distributed amongst control-, low level and high level treatment group.

Table 14 presents the non-neoplastic changes observed in 10 female and 10 male rats from the high dose- and control group killed at 28 months. The observed pathoses were equally present in control- and treatment groups or occurring in a single animal. They are common findings in aged rats of this strain and therefore were not considered to be induced by the administration of maleic hydrazide.

Incidence, site and type of tumours in rats that died or were killed in extremis or at the end of the experiment and the number of tumour bearing rats are presented in table 15. The most common neoplasms were benign phaeochromacytomas of the adrenals, chromophobe adenomas of the pituitary gland, mammary fibroadenomas and subcutaneous fibrosarcomas. The animals exhibited no tumours of a particular, unusual type. All tumours listed are common neoplasms in the

strain of rats used. Although some differences in tumour incidence occurred between control- and treatment groups, there was no indication that these differences were related to the feeding of the test-compound.

Whether treatment with maleic hydrazide could be associated with an earlier appearance of tumours was analysed also. From the data it is clear that maleic hydrazide does not shorten the latency period of neoplastic development (table 16).

DISCUSSION

A carcinogenicity experiment with rats of the F_1-generation during 28 months was carried out. In order to investigate the carcinogenic properties of maleic hydrazide, the compound as free acid was administrated into the diet at levels of 0, 1.0 and 2.0%. Special attention was given that the test compound used contained less than 1.5 mg hydrazine as impurity per kg product. In addition a number of toxicological observations were made concerning behaviour, food- and water-intake, mortality, growth and several haematological and clinical-chemical parameters in blood.

Animals killed after one year and after 28 months, as well as the animals that died between time were examined for the presence of tumours and/or other non-neoplastic changes. The results of the study showed that the tested concentrations of maleic hydrazide gave an initial growth reduction in female rats, an increased water-consumption and a consistently increased urine protein value associated with enhanced urinary protein/creatinin ratios in both sexes. These latter findings are indications for an impaired kidney function. However the kidney weights were normal and histopathological examination did not show morphological changes after 12 and 28 months. In the range-finding experiment carried out by Kroes et al (1974) early degenerative kidney lesions and kidney dysfunctions were found in rats fed 4% of maleic hydrazide for two weeks. It is clear that in this experiment the kidneys are the target organ and levels of 1% and higher induce renal dysfunction.

All other parameters which were studied did not show significant changes that are related to the treatment with maleic hydrazide.

As mentioned already this 28 month experiment, in principal, was carried out to study the carcinogenicity of maleic hydrazide. The tumour incidence, location and type of neoplastic changes did not reveal any treatment-related difference between test groups and control group. Moreover analysis of the time of the appearance of the tumours in the various groups did not prov_de

evidence for a shortening of the latency period under influence of maleic hydrazide.

These results are confirmed by an experiment with mice carried out in about the same period at the IARC, with the same batch of maleic hydrazide. The results of this study were also negative. The results of both experiments are in most cases comparable with the results of experiments carried out in the past. A longterm toxicity experiment (Food Research Laboratories Inc. 1954) with 5% in the diet was negative. An oral carcinogenicity study with 1% of maleic hydrazine (Barnes et al 1957) in rats and mice was also negative. In another carcinogenicity study with 7 days old mice, which were administrated by stomach tube daily 1000 mg maleic hydrazide/kg bodyweight for 3 weeks, after which the animals received 0.3% in the diet for 18 months, a negative result was found (Innes et al 1969).

Furthermore Barnes et al 1957, Hunter et al 1973, carried out studies with rats and/or mice respectively, in which the compound was administrated subcutaneously. Barnes and coworkers gave weekly applications of 500 mg/kg bodyweight for 100 weeks while Hunter and coworkers gave twice weekly injections of 2 mg maleic hydrazide per animal for 65 weeks. Thereafter these animals were kept for another 39 weeks. The first experiment (Barnes et al 1957) was negative while in the second experiment (Hunter et al 1973) a slight increase in subcutaneous tumours at the site of injection was found, when the compound was dissolved in arachis oil, but negative results were seen when the compound dissolved in water.

In the study of Epstein et al (1967) and Epstein and Mantel (1968), an experiment with subcutaneous injections of maleic hydrazide acid is described with a total dose of 3 mg in drinkingwater or 55 mg in tricaprylin given in 4 divided doses on day 1, 7, 14 and 21 of age. In this study an increased incidence of liver cell tumours was found. It should be emphasized that the maleic hydrazide used in this study contained 0.4% of the potent liver carcinogen hydrazine (see IARC monograph 1974). Therefore the significance of the liver cell tumours obtained in this study can hardly be assigned to maleic hydrazide.

To investigate the possible mutagenic potency of maleic hydrazide a dominant lethal study was carried out by Epstein et al (1972). Mice were treated during one cycle of spermatogenesis with a total dose upto 1000 and 2000 mg maleic hydrazide/kg bodyweight orally and intraperitoneally respectively. The results indicate that treatment with maleic hydrazide did not affect the calculated mutation index. Maleic hydrazide also failed to increase the incidence

of micronuclei in mice treated intraperitoneally with 200 mg/kg bodyweight (Chaubey et al. 1978).

From mutagenicity studies in lower organisms as several mutant tester strains of Salmonella typhimurium, bacteriophages and Drosophila melanogaster, the results dc not provide evidence that maleic hydrazide possesses mutagenic activity (Anderson et al, 1972; Mc Cann et al, 1975; Mc Cann and Ames, 1976; Nasrat, 1965).

From the results of the present experiment and of that performed at IARC it becomes clear that pure maleic hydrazide (99% pure) containing only low levels of hydrazine ($\leqslant$ 1.5 mg/kg product) is not carcinogenic. All the older experiments confirm this statement except the positive experiment of Epstein and coworkers who tested the compound containing the carcinogen hydrazine as impurity. Also the mutagenicity experiments support the negative results of the carcinogenicity studies.

Because maleic hydrazide should not be considered being a carcinogen, the other toxicological parameters are of importance. From the results available it is clear that 1% in the diet is still not a no-effect level. For this reason it is suggested that an additional short-term experiment should be carried out in order to establish a no-effect level on the basis of the kidney function. Furthermore it is known that in metabolic experiments with animals it was found that maleic hydrazide does not appear to be extensively metabolised by mammals (Mays et al, 1968). The compound is rapidly excreted unchanged with the urine. Liver microsomes isolated from phenobarbital induced rats which are capable of metabolizing a large number of xenobiotic substances, fail to produce any degradation products of maleic hydrazide such as maleic acid, fumaric acid, CO_2 or the metabolite hydrazine (Nelson and Kearney, 1977).

At last it could be questioned, in the case if maleic hydrazide residues are present on crops such as onions and potatoes, what will happen with these residues during household cooking and frying. It was found that maleic hydrazide did not decompose during cooking and frying under normal conditions (RIV 1955 and WHO 1977) and that pyrolysis of the compound at different temperatures did not produce hydrazine (Harke et al 1973).

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Table 1: Cumulative mortality of rats fed maleic hydrazide at dietary levels of 0, 1.0 and 2.0% for 28 months.

mortality after months	dietary level of maleic hydrazide					
	females			males		
	control	1%	2%	control	1%	2%
number of animals per group	55	55	65	55	55	65
3	0	0	0	0	0	0
6	2	2	1	0	0	0
9	4	5	5	1	0	0
12	10*	10*	11*	8*	8*	5*
15	13	12	14	9	10	8
18	15	16	16	12	10	10
21	20	20	22	19	14	19
24	23	27	31	29	17	33
28	29	41	42	34	26	44
mortality in percentage	50%	74%	64%	62%	47%	67%

* After 12 months of each group and sex five animals were killed. These are included in the mortality figures.

Table 2: Initial body-weight and weight-gain of rats fed maleic hydrazide in the diet for 28 months.

dietary levels of MH	Initial bodyweight and weightgain in g		
	control	1.0 %	2.0 %
females			
initial bodyweight	76 (55)	75 (55)	73** (65)
week 6	108 (55)	101** (55)	102** (65)
week 12	143 (55)	130*** (55)	133** (65)
week 20	168 (55)	151*** (55)	154** (64)
week 40	204 (52)	187* (50)	190* (63)
week 52	208 (45)	196 (45)	192* (55)
week 60	215 (45)	194 * (45)	200 (54)
week 80	249 (41)	230 (39)	229 (50)
week 100	246 (36)	231 (33)	228 (39)
week 104	237 (33)	227 (32)	224 (35)
week 120	233 (28)	219 (15)	211 (24)
males			
initial bodyweight	83 (55)	83 (55)	83 (65)
week 6	202 (55)	197 (55)	192* (65)
week 12	278 (55)	271 (55)	268 (65)
week 20	322 (55)	313 (55)	312 (65)
week 40	389 (54)	383 (55)	381 (65)
week 52	394 (47)	396 (48)	383 (60)
week 60	409 (47)	408 (47)	390 (58)
week 80	454 (43)	459 (46)	434 (56)
week 100	440 (31)	453 (41)	411 (41)
week 104	428 (29)	427 (38)	393 (35)
week 120	392 (22)	392 (30)	371 (23)

between brackets: number of animals still living

* = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$

Table 3: Mean food consumption of rats fed maleic hydrazide in the diet for 28 months.

dietary level of MH	mean food consumption in g/day during the first 12 weeks		
	control	1.0%	2.0%
females			
week 1	9.6	9.7	10.0
week 2	11.0	11.2	11.3
week 5	11.3	11.8	11.3
week 9	11.9	11.1	11.3
week 12	11.9	11.0	11.0
males			
week 1	11.5	12.1	12.2
week 2	14.0	14.8 *	14.9
week 5	18.1	17.9	17.7
week 9	17.6	17.4	18.4
week 12	18.4	17.7	18.7

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$

Table 4: Mean water consumption per rat per day in g at week 18 and 25

dietary level of MH	control	1.0 %	2.0 %
females			
week 18	16.6	20.5	27.0***
week 25	15.7	19.4	29.7**
males			
week 18	19.4	23.3	31.0**
week 25	18.3	21.1	30.9**

* $P \leq 0.05$ ** $P \leq 0.01$ and *** $P \leq 0.001$

Table 5: Mean haematological values of rats fed maleic hydrazide in the diet for 28 months

haematological values	dietary level of MH	mean haematological values at the end of month 6,12,24,28							
		females				males			
		month 6	month 12	month 24	month 28	month 6	month 12	month 24	month 28
haemoglobin in mmol/l	control	9.3	9.0	8.7(9)	8.1(26)	9.5	9.4	8.7(8)	8.3(16)
	1.0%	9.4	9.3	7.9	8.1(14)	9.5	9.4	9.3(10)	9.0(24)
	2.0%	8.7*	9.2	8.9	7.8(22)	9.7	9.6	8.8(10)	8.2(17)
haematocrit l/l	control	0.439	0.446	0.421	0.390	0.449	0.461	0.424	0.408
	1.0%	0.441	0.455	0.386	0.399	0.454	0.466	0.449	0.434
	2.0%	0.415*	0.450	0.432	0.389	0.461	0.468	0.423	0.406
erythrocytes (10^{-12}/l)	control	7.47	7.71	6.99	6.37	7.87	8.41	7.29	7.24
	1.0%	7.30	7.90	6.54	6.28	7.99	8.39	7.83*	7.51
	2.0%	7.08	7.87	7.20	6.32	8.17	8.53	7.43	7.25
leucocytes (10^{6}/l)	control	7670	9220	11022	12250	11580	13480	14900	15619
	1.0%	7040	7830	14770	14307	10000	11710	13850	13100
	2.0%	8520	9150	12170	12991	9630	11170	15480	13641
MCV/fl	control	58.8	58.0	60.4	61.8	56.8	54.9	58.3	56.4
	1.0%	60.3	57.6	59.6	64.1	57.1	55.7	57.4	58.0
	2.0%	58.5	57.1	60.4	61.6	56.5	55.0	57.2	55.8
MCH in a mol	control	1250	1169	1240	1269	1209	1123	1191	1139
	1.0%	1291*	1172	1209	1294	1185	1117	1183	1192
	2.0%	1229	1175	1250	1227	1190	1125	1197	1123
MCHC mmol/l	control	21.2	20.2	20.5	20.6	21.2	20.4	20.5	20.2
	1.0%	21.4	20.3	20.3	20.2	20.9	20.1	20.6	20.6
	2.0%	21.0	20.6*	20.7	19.9*	21.1	20.4	20.8	20.2

average of 10 animals except mentioned between brackets

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$

Table 6: Mean haematological values (differential white cell counts) of rats fed maleic hydrazide in the diet for 28 months.

differential white cell counts	dietary level of MH	Mean differential white cell counts at the end of month 6, 12, 24 and 28							
		females				males			
		month 6	month 12	month 24	month 28	month 6	month 12	month 24	month 28
baso-philic cells in %	control	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.1
	1.0%	0.1	0.2	0.3	0.1	0.2	0.1	0.3	0.3
	2.0%	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1
eosino-philic cells in %	control	1.2	0.9	0.8	0.5	1.6	1.3	0.7	0.6
	1.0%	1.5	0.9	0.7	0.6	1.3	1.0	1.0	0.9
	2.0%	1.1	0.6	0.5	0.4	1.1	1.5	1.1	0.5
neuro-trophi-lic cells in %	control	9.6	14.7	34.2	35.6	8.0	11.8	25.6	36.2
	1.0%	8.1	14.7	43.8	39.6	8.8	10.2	23.0	28.2*
	2.0%	13.5	15.8	35.5	40.1	8.0	9.6	31.6	34.8
lympho-cytes in %	control	85.4	79.5	56.7	56.6	87.7	82.9	67.2	54.7
	1.0%	86.4	79.9	48.1	51.4	86.2	84.7	70.7	62.2
	2.0%	81.1	78.9	58.3	51.1	88.8	84.2	58.8	56.9
monocy-tes in %	control	3.7	4.7	8.1	7.2	2.6	3.8	6.4	8.4
	1.0%	3.9	4.3	7.1	8.3	3.5	4.0	5.0	8.4
	2.0%	4.2	4.6	5.6*	8.2	2.0	4.6	8.5	7.7

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$

Table 7: Mean clinical chemical values of rats fed maleic hydrazide in the diet for 28 months

clinical chemical values	dietary level of MH	mean clinical chemical values at the end of months 6,12,24					
		females			males		
		month 6	month 12	month 24	month 6	month 12	month 24
Alk.Pase U/l	control	91±21	85±28	88±35	106±28	102±12	136±41
	1.0%	81±10	48±10	99±53	106±19	88±8*	80±19
	2.0%	84±20	59±24	98±17	104±21	104±18	128±91
SGPT U/l	control	18±5	26±16	29±11	11±2	17±8	29±15
	1.0%	18±8	18±7	36±3	13±2	14±6	23±7
	2.0%	16±4	26±9	37±15	15±2	19±3	23±10
SGOT U/l	control	98±17	67±21	136±60	101±23	55±14	142±72
	1.0%	90±15	48±10	182±89	80±20	46±17	122±22
	2.0%	77±9*	59±24	157±41	69±12*	44±16	106±16
urea mmol/l	control	7.9±0.6	5.7±0.9	5.1±1.1	7.3±0.8	5.5±0.5	5.5±1.4
	1.0%	7.9±1.6	7.0±1.4	6.8±0.8	6.4±0.4*	5.8±0.9	5.3±0.8
	2.0%	6.4±0.9**	7.3±1.0*	7.0±1.3	6.3±0.7*	5.5±0.8	5.6±0.7
glucose mmol/l	control	6.8±0.6	6.5±0.6	6.8±0.4	6.9±0.6	7.2±0.9	6.3±0.8
	1.0%	6.8±0.7	7.0±0.9	7.9±0.6*	7.2±0.3	7.7±0.1	5.8±0.7
	2.0%	5.8±0.9	5.2±1.2	5.8±0.5	7.4±0.5	8.3±0.3*	5.5±1.0

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$

Table 8: Urinalyses (frequency table) of rats fed maleic hydrazide in the diet for 28 months

sex	dietary level of MH	semi-quantitative values in the urine at the end of 3, 6, 12 and 24 months													
		pH			protein					ketones		blood			
		5.5	6	7	±	+	++	+++	++++	-	±	-	+	++	+++
					after 3 months										
females	control	-	8	-	1	6	1	-	-	8	-	8	-	-	-
	1.0%	-	8	-	-	7	1	-	-	8	-	8	-	-	-
	2.0%	2	6	-	-	3	5	-	-	8	-	7	-	1	-
males	control	-	7	1	-	5	3	-	-	8	-	8	-	-	-
	1.0%	-	8	-	-	1	4	3	-	8	-	8	-	-	-
	2.0%	-	8	-	-	2	5	1	-	8	-	8	-	-	-
					after 6 months										
females	control	2	6	-	3	4	1	-	-	8	-	8	-	-	-
	1.0%	1	7	-	3	4	1	-	-	8	-	6	2	-	-
	2.0%	1	6	-	1	6	-	-	-	7	-	7	-	-	-
males	control	3	5	-	-	5	3	-	-	8	-	7	1	-	-
	1.0%	1	6	-	-	3	4	-	-	7	-	6	1	-	-
	2.0%	3	3	-	-	4	2	-	-	6	-	5	-	1	-
					after 12 months										
females	control	4	4	-	1	4	3	-	-	8	-	8	-	-	-
	1.0%	2	6	-	1	5	2	-	-	8	-	8	-	-	-
	2.0%	4	4	-	1	3	4	-	-	7	1	6	1	-	1
males	control	-	6	-	-	-	6	-	-	6	-	6	-	-	-
	1.0%	1	7	-	-	3	4	1	-	8	-	8	-	-	-
	2.0%	1	7	-	-	2	4	2	-	8	-	7	-	-	1
					after 24 months										
females	control	2	6	-	-	2	6	-	-	8	-	4	2	2	-
	1.0%	2	6	-	-	3	5	-	-	8	-	2	3	2	1
	2.0%	1	7	-	1	3	4	-	-	8	-	3	-	4	1
males	control	-	7	-	-	-	7	-	-	6	1	4	1	2	-
	1.0%	1	7	-	-	2	3	2	1	8	-	6	-	2	-
	2.0%	-	7	-	-	-	6	-	1	7	-	-	1	5	1

-, ±, +, ++, +++, ++++ = resp. none, trace, some, moderate, considerable and high amounts

Glucose and bilirubin: all urines negative

Table 9: Osmolality values of urine of rats fed maleic hydrazide in the diet for 28 months.

dietary level of MH	osmolality in m. osmol/l after 26 months	
	females	males
control	2714 ± 247	2812 ± 653
1.0 %	2692 ± 354	2550 ± 536
2.0 %	2180 ± 1003	2034 ± 770

Table 10: Urine-protein and creatinin values and serum PSP-clearance of rats fed maleic hydrazide for 28 months

sex	dietary level of MH	PSP in serum mg/l	protein g/l	creatinin (g/l)	protein/creatinin ratio
			after 6 months		
females	control	-	5.0± 1.0	2.72±0.82	1.9±0.3
	1.0%	-	20.5±11.8***	2.01±0.70	10.0±3.3***
	2.0%		14.9± 6.9**(7)	1.87±0.30*	9.5±1.6***
males	control	-	6.3± 1.0	3.59±0.48	1.8±0.3
	1.0%	-	19.5± 4.9***(7)	2.75±0.74	7.0±1.4***
	2.0%	-	18.0± 2.3***(6)	2.49±0.66	7.6±1.6***
			after 12 months		
females	control	12.8±3.1	5.0± 1.1	3.1±0.9	1.7±0.3
	1.0%	14.0±3.0	18.2± 2.1***	2.5±0.9	8.2±2.9***
	2.0%	13.8±1.1	22.4±10.2***	2.6±0.6	8.6±2.9***
males	control	17.4±4.1	7.1± 0.9(5)	4.3±0.5	1.6±0.1
	1.0%	17.1±2.1	19.0± 4.2***(7)	3.4±0.3**	5.6±1.3***
	2.0%	18.0±2.4	26.1± 8.0***	3.1±0.8**	8.9±2.5***

between brackets number of animals different from 8

* P= $\leqslant$ 0.05 , ** P= $\leqslant$ 0.01 and *** P= $\leqslant$ 0.001

Table 11: Absolute organ weight in mg of rats fed maleic hydrazide for 12 months

dietary level of MH	control	1.0 %	2.0%
females			
body wt.	277 g	236 g	256 g
heart	754	733	747
brain	1898	1795	1846
liver	7830	6480	7760
kidneys	1743	1527	1662
spleen	524	462	599
thymus	146	287	186
adrenals	50	50	44
thyroid	22	22	30
pituitary	16	16	13
ovaries	51	37	56
males			
body wt.	467 g	389 g	458 g
heart	918	930	961
brain	1961	1952	2036
liver	10180	9410	11190
kidneys	2364	1916	2399
spleen	667	603	793
thymus	178	194	185
adrenals	47	44	47
thyroid	40	31	36
pituitary	12	11	13
testes	3173	2463	3146

average of 5 animals

Table 12: Mean organ/bodyweight ratios of rats fed maleic hydrazide for 12 months

dietary level of MH	control	1.0%	2.0%
<u>females</u>			
heart	0.274	0.310*	0.292
brain	0.700	0.767	0.726
liver	2.83	2.74	3.04
kidneys	0.636	0.653	0.648
spleen	0.191	0.197	0.235
thymus	0.052	0.133	0.072*
adrenals	0.018	0.022*	0.017
thyroid	0.008	0.009	0.009
pituitary	0.006	0.007	0.005
ovaries	0.018	0.016	0.023
<u>males</u>			
heart	0.197	0.247*	0.212
brain	0.423	0.571	0.451
liver	2.18	2.46	2.45*
kidneys	0.507	0.520	0.524
spleen	0.131	0.160	0.174**
thymus	0.038	0.046	0.040
adrenals	0.010	0.013	0.010
thyroid	0.009	0.008	0.008
pituitary	0.003	0.003	0.003
testes	0.681	0.649	0.695

average of 5 animals

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P < 0.001$

Table 13 : Summary of the histo-pathological findings in rats fed maleic hydrazide for 12 months

dietary level of MH	control		1.0 %		2.0 %	
sex	females	males	females	males	females	males
BRAINS examined at 3 levels	5	5	4	5	5	5
Focal dystrophic calcification				1		
HEART examined	5	5	5	5	5	5
Slight myocard degeneration					1	
LUNG examined	5	4	5	5	5	5
Histiocytosis					1	
LIVER examined	5	5	5	4	4	5
Bile duct proliferation	1	1	1	1	1	2
Focal necrosis			1		1	
KIDNEYS examined	5	5	5	4	5	5
Calcium deposits in C-M regio			1			
SPLEEN examined	5	5	5	5	5	5
Slight haemosiderosis		4	1	4	4	2
Moderate haemosiderosis			1		1	3
Extensive haemosiderosis	3		2	1		
Atrophy of lymphoid tissue	1					
PITUITARY examined	5	5	5	4	5	4
Chromophobic adenoma	1		1			
Hyperplasia of basophilic cells		1				
Hyperplasia of intermedia					1	
THYROID examined	5	5	5	4	5	4
Parafollicular cell proliferation	1		2			1
slight activation				1		
THYMUS examined	3	5	4	2	5	4
Involution	3	4	2	2	2	3
Thymosarcoma			2			
PANCREAS examined	5	5	4	5	4	5
ADRENALS examined	5	5	5	4	5	4
irregular Z glomerulosa		1				
Hyperplasia cortex	2	2	3	2		1
heamorrhagia of the cortex				1		

Table 13 continued: Summary of the histopathological findings in rats fed maleic hydrazide for 12 months

dietary level of MH	control		1.0%		2.0%	
sex	females	males	females	males	females	males
Hyperplastic medullary cells					1	
Phaeochromocytoma					1	
OVARIES examined	5		5		5	
Senile atrophy	2		3		2	
Enlarged C.luteum	1					
UTERUS examined	5		5		5	
Hyalinization of stroma	2				1	
MAMMA adenoma			1			
TESTES examined		5		5		5
Slight atrophy				1		
Atrophy (one side)				1		
Atrophy (both sides)						1
PROSTATE examined		5		5		5
Prostatitis		1				
LYMPH-NODES examined	4	4	5	5	5	5
Increased number of fat loaded macrophages	4	3	3	2	4	5
MESENTERIC periarteritis	1				1	
SALIVARY GLAND examined	5	5	5	5	5	5
STOMACH examined	5	5	5	5	5	5
Focal necrosis (pylorus)	1					
INTESTINE examined at 3 levels	5	5	5	5	5	5
Increased cellularity villi	4	5	4	5	5	4
BLADDER examined	5	4	5	5	4	5
SPINAL CORD at 3 levels	5	5	5	5	4	5
PERIPHERAL NERVES (N.Isch) ex.	5	5	4	4	4	5
MUSCULATURE examined	4	5	5	5	5	5
SKIN examined	4	4	4	5	5	5

Table 14: Non-neoplastic histopathological findings in rats fed maleic hydrazide and the number of animals showing the observed lesions for 28 months

dietary level of MH	control		1.0 %		2.0%	
sex	females	males	females	males	females	males
number of animals examined	10	10	-	-*	10	10
BRAIN examined	10	10	-	-	10	10
1. calcium deposits in vessels	0	0	-	-	0	
2. haemocysts	1	0	-	-	0	0
3. small haemorrhage	0	1	-	-	0	0
HEART examined	9	9	-	-	9	10
1. focal myocarditis	0	1	-	-	0	1
2. focal myodegeneration and myofibrosis	1	3	-	-	1	5
LUNG examined	10	10	-	-	10	10
1. histiocytosis	3	1	-	-	2	2
2. interstitial pneumonia	3	2	-	-	1	5
3. lobular pneumonia	1	0	-	-	1	0
LIVER examined	9	10	-	-	10	10
1. hepatitis	0	0	-	-	0	1
2. bileduct proliferation and hyperplasia (tubular)	4	6	-	-	4	7
3. cystic bileduct proliferation	1	1	-	-	0	0
4. cholangiofibrosis	0	1	-	-	2	1
5. pericholangitis	1	5	-	-	2	5
6. vacuolation of periportal livercells	0	1	-	-	1	0
7. focal livercell necrosis	0	2	-	-	0	0
8. areas of cellular alterations	1	2	-	-	0	7
KIDNEYS examined	10	10	-	-	10	10
1. glomerulonephrosis	6	5	-	-	4	4
2. dilated tubules	0	0	-	-	0	1
3. glomerulo nephritis	0	3	-	-	1	0
4. hyperplasia of pyelum epithelium	0	1	-	-	0	0
5. deposition of pigment in tubular epithelial cells	0	0	-	-	0	2
SPLEEN examined	10	10	-	-	9	10
1. haemosiderosis a) slight degree	3	1	-	-	0	0
b) moderate degree	0	2	-	-	1	2
c) marked degree	0	1	-	-	1	0

Table 14 continued: Non-neoplastic histopathological findings in rats fed maleic hydrazide and the number of animals showing the observed lesions for 28 months

dietary level of MH	control		1.0%		2.0%	
sex	females	males	females	males	females	males
2. haematopoiesis						
a) slight degree	1	0	-	-	3	2
b) moderate degree	4	0	-	-	2	2
c) marked degree	1	1	-	-	0	2
3. reticulum cell hyperplasia	0	1	-	-	0	0
4. marked depletion of lymphocytes	0	0	-	-	1	0
PITUITARY GLAND examined	7	8	-	-	2	7
1. hyperplasia of chromophobe cells	0	2	-	-	0	1
THYROID examined	7	10	-	-	8	9
1. γ cell hyperplasia a) type a	0	3	-	-	0	2
b) type b	0	0	-	-	1	0
c) type c	2	2	-	-	0	1
THYMUS examined	8	7	-	-	9	7
1. marked involution	7	7	-	-	7	6
PANCREAS examined	10	10	-	-	10	10
1. islet cell hyperplasia	0	0	-	-	0	1
2. polyarteritis	0	0	-	-	1	0
ADRENALS examined	10	10	-	-	8	10
1. cortical hyperplasia	4	3	-	-	1	4
2. vacuolation of cortical cells	1	0	-	-	0	0
3. congestion and hyperaemia in medulla	1	0	-	-	0	0
OVARIES examined	10	-	-	-	9	-
1. senile atrophy	4	-	-	-	4	-
2. interstitial cell hyperplasia	1	-	-	-	0	-
TESTES examined	-	9	-	-	-	10
1. periarteritis	-	1	-	-	-	1
2. unilateral degenerative atrophy	-	2	-	-	-	3
3. bilateral degenerative atrophy	-	2	-	-	-	2
UTERUS examined	10	-	-	-	10	-
1. fibromatous polyp	1	-	-	-	1	-
2. hyaline stromal degeneration	4	-	-	-	6	-
3. cystic endometrium	1	-	-	-	0	-
4. glandular hyperplasia	1	-	-	-	4	-
PROSTATE examined	-	9	-	-	-	9
1. prostatitis	-	1	-	-	-	1
2. inflammatory changes	-	0	-	-	-	1

Table 14 continued: Non-neoplastic histopathological findings in rats fed maleic hydrazide and the number of animals showing the observed lesions for 28 months

dietary level of MH	control		1.0 %		2.0 %	
sex	females	males	females	males	females	males
MESENTERIC LYMPH-NODE examined	10	8	-	-	8	7
1. increased number of macrophages in medulla	7	4	-	-	5	3
STOMACH examined	10	10	-	-	10	10
1. ulcer	0	0	-	-	1	0
2. papillomatous hyperplasia	0	0	-	-	0	1
SMALL INTESTINES examined	10	10	-	-	10	10
1. enteritis	0	3	-	-	3	3
SALIVARY GLAND examined	10	10	-	-	10	10
1. inflammation	0	0	-	-	1	0
2. ductal metaplasia	0	1	-	-	1	1
SPINAL CORD examined	9	9	-	-	4	5
1. demyelination a) slight degree	3	1	-	-	2	-
b) moderate degree	2	8	-	-	1	4
c) marked degree	0	1	-	-	1	0
NERVUS ISCHIADICUS examined	9	10	-	-	7	9
1. degeneration and fibrosis	7	9	-	-	5	7
SKELETAL MUSCLE examined	10	10	-	-	9	10
1. neurogenic atrophy	2	6	-	-	3	5

* not examined

N.B. Popliteal lymph-nodes and urinary bladder did not show abnormalities at all

Table 15: Incidence, site and type of tumour in the different groups of rats fed maleic hydrazide for 28 months (survivors and non-survivors together)

Site and type of tumours	maleic hydrazide in the diet					
	females			males		
	control	1.0%	2.0%	control	1.0%	2.0%
Initial number of rats	55	55	65	55	55	65
effective number of rats	53	50	61	54	53	64
number of rats showing too advanced autolysis	2	5	4	1	2	1
number of rats killed at 12 months						
a) without tumour	3	3	4	5	5	5
b) with tumour	2	2	1	0	0	0
number of surviving rats examined	26	14	23	21	28	22
number of non-surviving rats examined	27	36	38	33	25	42
total number of tumour bearing rats	33	35	42	41	32	41
total number of primary tumours	59	48	63	57	46	56
total number of rats having one tumour	13	25	25	24	24	29
total number of rats having two tumours	12	7	13	15	8	10
total number of rats having three or more tumours	6	3	4	1	2	2
A PITUITARY GLAND						
1 chromophobe adenoma	18	20	24	13	14	15
2 chromophobe carcinoma	1	1	1	-	1	1
3 eosinophilic carcinoma	-	-	-	1	1	-
4 pars intermedia adenoma	-	-	1	-	-	1
B THYROID						
1 parafollicular cell carcinoma	1	-	2	-	2	2
2 follicular adenoma	-	1	1	-	-	1
C ADRENALS						
1 benign phaeochromocytoma	4	7	4	11	8	11
2 ganglioneuroma	1	1	-	-	-	-
3 malign phaeochromocytoma	-	-	1	4	-	4
4 cortical adenoma	-	-	-	2	-	1
5 fibroma	-	1	-	-	-	-

Table 15 continued: Incidence, site and type of tumour in the different groups of rats fed maleic hydrazide for 28 months (survivors and non-survivors together)

	Site and type of tumours	maleic hydrazide in the diet					
		females			males		
		control	1.0%	2.0%	control	1.0%	2.0%
D	MAMMARY GLAND						
1	fibro adenoma	16	3	13	1	3	1
2	adenoma	2	2	1	-	-	-
3	adenocarcinoma	2	1	-	-	-	-
E	SKIN AND SUBCUTIS						
1	fibroma	1	-	2	5	5	4
2	fibrosarcoma	5	2	-	4	-	2
3	squamous cell carcinoma	1	-	1	2	-	-
4	lipoma	-	-	-	-	2	-
5	basal cell carcinoma	-	-	-	1	-	1
6	trichoepithelioma	-	-	-	-	1	-
F	ABDOMINAL CAVITY						
1	anaplastic carcinoma	-	-	-	1	1	-
2	fibrosarcoma	2	1	1	-	-	1
3	unclassified tumour	-	1	-	-	-	-
4	osteosarcoma	-	-	-	-	-	2
G	BRAIN						
1	granularcell myoblastoma	-	-	-	2	-	-
2	ependymoma	-	-	-	1	1	-
3	astrocytoma	-	-	-	1	-	-
4	glioma	-	1	-	-	-	-
H	SPLEEN						
1	haemangioma	1	-	-	-	-	-
2	fibrosarcoma	-	-	-	-	1	-
I	COLON						
1	papillary carcinoma	-	-	1	-	-	-
J	THYMUS						
1	lymphoma	1	-	-	-	-	-
2	lymphosarcoma	-	2	1	-	-	-
K	PANCREAS						
1	isletcell tumour	1	-	-	-	-	1
L	LIVER						
1	bileduct adenoma	-	-	-	1	-	-
2	cholangiocystadenoma	-	-	-	-	1	-

Table 15 continued: Incidence, site and type of tumour in the different groups of rats fed maleic hydrazide for 28 months (survivors and non-survivors together).

Site and type of tumours	maleic hydrazide in the diet					
	females			males		
	control	1.0%	2.0%	control	1.0%	2.0%
3 neoplastic nodule	-	1	-	-	-	2
M URINARY BLADDER						
1 papilloma	-	1	1	-	-	-
2 adenoma	-	-	1	-	-	-
N HAEMATO-LYMPHOPOIETIC SYSTEM						
1 lymphocytic leukemia	-	1	2	6	2	3
2 lymphosarcoma in cervical lymphnode	-	-	-	1	-	-
O LEG						
1 fibrosarcoma	-	1	1	-	1	2
2 osteosarcoma	1	-	1	-	1	-
P PLEURAL CAVITY						
1 ganglioneuroma	-	-	-	-	1	-
2 osteosarcoma	-	-	1	-	-	-
♀ UTERUS						
1 fibromatous polyp	1	-	-			
2 fibrosarcoma	-	-	1			
R OVARIES						
1 granulosa cell tumour	-	-	1			
S PROSTATE						
1 adenoma				-	-	1

Table 16: Incidence, (number of rats with tumour*/number of rats observed), type and site of tumours** observed, in rats fed maleic hydrazide for 28 months, during the following periods (months):

Maleic hydrazide in the diet and sex	0 - 12	12 - 14	14 - 16	16 - 18	18 - 20	20 - 22	22 - 24	24 - 26	26 - 28
Control, females	A1 2/10 D1	0/0	0/0	E2 2/2 A1 F2	D1 2/2 D1	D1 1/2	02 2/3 D1 A1	0/0	A1 15 E1 1 24/32 A2 1 E2 4 B1 1 E3 1 C1 4 F2 1 C2 1 H1 1 D1 11 J1 1 D2 2 K1 1 D3 2 Q1 1
Control, males	N1 3/8 N1 N1	0/1	E2 1/1	N1 2/3 N1	F1 2/3 A1	A1 5/6 A1 C1 C3 E3 G1 N1 N2	A1 2/4 C1	A3 5/5 C1 C3 E1 E2 E6 G2	A1 9 G1 1 20/24 C1 8 G3 1 C3 2 L1 1 C4 2 D1 1 E1 4 E2 2 E3 1

Table 16 continued: Incidence, (number of rats with tumour* /number of rats observed), type and site of tumours** observed, in rats fed maleic hydrazide for 28 months, during the following periods (months):

maleic hydrazide in the diet and sex	0 - 12	12 - 14	14 - 16	16 - 18	18 - 20	20 - 22	22 - 24	24 - 26	26 - 28
1.0%, females	J2 2/10 J2	0/0	0/2	N1 1/3	A1 2/3 A1	A1 1/1	A1 3/3 A1 E2	A1 5 C1 2 C2 C6 D1 F2 G4 01	A1 10 F3 1 16/20 A2 1 L3 1 B2 1 M1 1 C1 5 D1 2 D2 2 D3 1 E2 1
1.0%, males	02 1/7	01 1/1	0/0	0/0	0/2	A1 2/2 C1	A3 4/4 E1 E1 G2	A2 1/2	A1 13 E7 1 25/37 B1 2 F1 1 C1 7 H2 1 D1 3 L2 1 E1 3 N1 2 E4 2 P1 1

Table 16 continued: Incidence, (number of rats with tumour*/number of rats observed), type and site of tumours** observed, in rats fed maleic hydrazide for 28 months, during the following periods (months):

maleic hydrazide in the diet and sex	0 - 12	12 - 14	14 - 16	16 - 18	18 - 20	20 - 22	22 - 24	24 - 26	26 - 28
2.0%, females	C1 1/10	P2 2/2 A1	M1 1/2 D1	0/1	A1 1/2	A1 3 5/5 D1 1 E3 1 O2 1 Q2 1	A1 3 6/9 D1 1 F2 1 N1 2	A1 1 2/2 C3 1 D1 1	A1 15 D2 1 24/31 A2 1 E1 2 A5 1 I1 1 B1 2 J2 1 B2 1 M2 1 C1 3 O1 1 D1 9 R1 1
2.0%, males	0/5	F4 1/1	0/0	0/0	E2 4/7 E6 C1 N1	A1 2 2/4	A1 3 7/10 C1 1 C3 1 E1 1 N1 2	A2 1 4/6 B1 1 C1 1 E2 1 O1 1	A1 10 E1 3 23/31 A5 1 F2 1 B1 1 F4 1 B2 1 K1 1 C1 8 L3 2 C3 3 O1 1 C4 1 S1 1 D1 1

* a number of rats bearing two or more tumours ** tumour code from tumour incidence table