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Long-term wholesomeness study of autoclaved or irradiated pork in rats

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Summary

Six groups of rats were fed for 2½ year with either a standard diet or a diet supplemented with pork, which was autoclaved or irradiated. Between the five experimental groups (control autoclaved, autoclaved, control irradiated, high irradiated or low irradiated) no differences were detected in growth, food intake and mortality.

Data from biochemical examination of blood and urine did not reveal any treatment related changes. Alterations in the white blood picture were found intercurrently in different experimental groups, but were not due to any treatment.

The only effect on organ weight was a decrease in the thyroid weight of the standard group in compare to the five treated groups.

Macroscopic examination of the rats killed at the end of the experiment as well as of the intercurrently autopsied animals that died or were killed because of ill health, did not indicate that any gross changes were induced by the diet administered.

The histopathological changes seen in different groups were also seen in the control groups. Therefore these changes were not considered to be related to the treatment.

From the data on tumour appearance and incidence it was concluded that the number of tumours and the latency period was comparable in the various treated and control groups.

From this relay study no effects attributable to the irradiation levels could be established.

Introduction

In an attempt to develop an approach for the wholesomeness testing of irradiated food, a research program was undertaken under co-responsibility of the Institute of Atomic Sciences in Agriculture and the National Institute of Public Health in The Netherlands. Basic criteria are the wholesomeness (i.e. absence of adverse health effects or safety for consumption of irradiated foods from the toxicological point of view) of irradiated diets and the comparison of irradiation with other physical processes, such as cooking, smoking and heat treatment. In an earlier reproduction experiment rats were given feed which was either radiation sterilized (50 kGy[1]), autoclave sterilized (120°C, 15 minutes) or conventionally autoclave treated (110°C, 10 minutes). No effects were seen. With the offspring of these rats a 90-day toxicity test was carried out. In this experiment the litters were fed the same diets as the respective parent generation. No treatment related histopathological changes were observed (1).

In a second study the total diets of pigs were radiation sterilized and compared to autoclave sterilized diets. In this pig experiment a 3 generation study with 2 litters per generation was done. With the first litter of the first generation a subchronic experiment was carried out with 3 groups of pigs, which were given feed which was either untreated (control), radiation sterilized (50 kGy) or autoclave sterilized (10 minutes, 120°C) during 16 weeks. It was unlikely that changes in body weight gain, hematological parameters, organ weights, and histopathology were related to the treatment (2).

In the present study the meat of the pigs of the subchronic study (2), supplemented with the meat of some sows of the reproductive study (report in preparation), was used to carry out a long-term wholesomeness study with rats. This study was undertaken in order to test the wholesomeness of irradiated pork compared with autoclaved pork, another physical preservation process also based on heat treatment. The meat was derived from pigs fed control diet, autoclaved diet or 50 kGy irradiated diet. The pork was additionally preserved by supplemental nitrite at low (50 ppm) or high (200 ppm) dose level. According to Wierbicki and Heiligman (3) the amounts of nitrite and nitrate, in cured meats, can be substantially reduced by use of radiation. In the case of irradiation of meat only 50 ppm sodium nitrite was added to the curing pickled meat instead of the conventional 200 ppm. Nitrite has to be used in order to stabilize taste and colour and to inhibit formation of botulin toxin.

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1) Gy = Gray = 1 $J.kg^{-1}$

Use of heat for preservation of food is generally accepted to be safe in the light of the experience of long use. Irradiation treatment also involves the addition of energy to the food with consequent modifications of the food components. Toxicological aspects of food components which are modified by irradiation are aimed to be studied.

Materials and methods

Diets

Preparation of canned pork

The meat used in the experiment was derived from 3 groups of pigs given autoclaved (10 minutes, 120°C), irradiated (50 kGy) or conventional pig feed. The male and female pigs (body weight about 100 kg) were sacrified and deboned at the slaughter house at Twello. The meat was transported under refrigeration to CIVO-TNO Zeist, where it was defatted, cut into pieces of about 3x3x3 cm and mixed with 10% curing brine. Mixtures of 150-500 kg derived from the treated groups were left in a drain churn for 16 hours at a temperature of 6°C. Thereafter portions of about 20 kg were mixed under vacuum and canned in tins of 450 g. The composition of the curing brine for 50 and 200 ppm nitrite containing meat is given in table 1.

Pasteurization and irradiation

Pasteurization was carried out at 75°C in a water bath, until the centre of the cans had reached a temperature of 70°C. For every batch the temperature in 2 cans was measured with a thermocouple (4). The pasteurized canned pork was stored at -40°C, till transportation under refrigeration to the Institute for Atomic Sciences in Agriculture (ITAL) at Wageningen where the irradiation was performed.

The product temperature during the ^{60}Co isotopic source irradiation was -30°C. The irradiation dose received was 74 kGy or 37 kGy respectively (5). After irradiation the canned pork was stored at room temperature.

Sterilization

Sterilization was performed by autoclaving at a temperature of 110°C until a sterilization value (F_0)* of 0.9 - 1 was reached. Thereafter these cans were stored also at room temperature.

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*) F_0 value is the process value: number of minutes necessary to sterilize a product at 110°C.

Chemical analysis

After irradiation or sterilization by autoclaving 2 to 4 tins of cured pork of every batch were analysed for moisture (dried at 105^{o}C), fat (butyrometrically), protein (% nitrogen x 6.25), salt (% NaCl), nitrite (ppm $NaNO_2$) and phosphate (% P_2O_5).

In addition the percentage jelly set off was determined (table 2). These analyses were performed at the Central Institute for Nutrition and Food Research, Zeist.

Volatile N-nitrosamines in samples of pork were determined with GC-MS by the Laboratory for Chemical Food Analysis (6) (table 3).

Composition of the diets

The control rats, without pork in their diet, received the semi-purified standard diet Muracon SSP-Tox. The composition of this diet is given in table 3.

At the National Institute of Public Health (Bilthoven) the canned pork was mixed with animal diet in a weight ratio of 35%. Since the canned pork had a relatively high protein and salt content, a "supplemented diet" was used. The standard diet and the supplement diet (table 4) were specially compounded by Trouw and Co., Putten, The Netherlands.

At two time intervals (after 2 and 9 months) during the study the experimental diets were analysed by the "Rijks Landbouwproefstation", Maastricht, for the protein, fat (ether extract), crude fiber, moisture (80^{o}C, vacuo) and chloride content. The results are given in table 5. Due to the high moisture content of the pork (about 75%) the moisture content of the experimental diets of the groups 2 until 6 is relatively high in comparison to the normal standard diet, whereas the protein, fat, cellulose and chloride concentration is lower.

The various diets were stored under refrigeration. The rats were given fresh feed daily.

Animals and diets

Male (weighed between 318 - 358 g) and female (194 - 214 g) SPF-derived Wistar rats Riv:TOX(M) were obtained from the Institute's own breeding colony. They were housed under conventional conditions in wire cages, two animals per cage. Food and drinking water were supplied ad libitum. The rats were divided randomly among six groups of 12 males and 24 females. The animals were fed an experimental diet according to the following scheme:

1. Standard : SSP (semi-synthetic purified)-Tox standard rat feed.
2. "Control autoclaved": 35% autoclaved meat, derived from pigs fed untreated conventional feed, with 200 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
3. Autoclaved : 35% autoclaved meat, derived from pigs fed autoclaved conventional feed, with 200 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
4. "Control irradiated": 35% 37 kGy irradiated meat, derived from pigs fed untreated conventional feed, with 50 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
5. Irradiated (low) : 35% 37 kGy irradiated meat, derived from pigs fed 50 kGy irradiated conventional feed, with 50 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
6. Irradiated (high) : 35% 74 kGy irradiated meat, derived from pigs fed 50 kGy irradiated concentional feed, with 50 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).

The six groups were given the 6 respective diets for two weeks. Thereafter each male was allowed to mate with two females for one week to produce a litter. With the offspring a chronic test was carried out.

Experimental design

Upon weaning six groups, each comprising 50 male and 50 female rats (except group 3: 49 and group 4: 42 females) were used. They were chosen randomly from the dams.

In this experiment the rats were fed the same experimental diet for a period of 2½ years as the respective parent generation. The rats were housed in wire cages, two animals per cage. Food and drinking water were given ad libitum.

The animals were weighed weekly during the first 12 weeks and every 8 weeks thereafter. Food intake was recorded in week 1, 2, 5, 9, 12, 20, 36, 52, 68, 84, 101 and 116.

Blood samples were taken for haematological investigations from 10 female and 10 male animals from each group after 13, 26, 52, 104 weeks and at the end of the study. The haemoglobin concentration, haematocrit value, concentration of erythrocytes and concentration and differential counts of leucocytes were determined. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated*. Serum urea concentration and activity of glutamic-pyruvic-transaminase (SGPT), glutamic-oxalacetic transaminase (SGOT) and alkaline phosphatase activity (Alk.Pase) were determined in 5 female and 5 male rats of each group after 26 and 104 weeks. The glucose concentration in the serum was estimated after 104 weeks. Semi-quantitative urinalysis of pH, protein, glucose, keton bodies, bilirubin and blood with Bililabstix was carried out after 26 and 104 weeks. After 104 weeks the concentration of creatinine and protein in urine was determined (7).

All animals were inspected regularly. Animals becoming moribund were autopsied. After 125 weeks the surviving animals were killed with carbon dioxide. Heart, brain, liver, kidneys, spleen, adrenals, thyroid, pituitary, coecum, uterus, ovaries, testes and prostate were dissected and weighed. All animals that died or were killed were autopsied and all organs were fixed in buffered 4% formalin (except brain in 10%) or Bouin solution for histopathological examination. The organs weighed as well as lungs, pancreas, mesenteric lymph nodes, salivary glands, stomach, duodenum, ileum, jejunum, colon, rectum, urinary bladder, spinal cord, n.ischiadicus and gross lesions were studied histopathologically.

Statistical analysis

Significancy of differences in group means - or geometric means - were established by the Student's t test (two-sided). In case of insufficient homogeneity of variances, the Welsh correction with respect to the degrees of freedom was applied.

* $0{,}01 \leq P < 0{,}05$ in comparison to group 2
** $0{,}001 \leq P < 0{,}01$ in comparison to group 2
*** $P < 0{,}001$ in comparison to group 2

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Results

Growth

The mean initial body weight and the weight gain recorded at week 6, 12, 36, 52, 68, 84, 100 and 116 is given in table 6. The growth of the rats is presented graphically in figure 1 for the females and for the males in figure 2. These data show that a diet containing 35% pork induced an increased growth compared to the standard rat diet. However, there were no clear differences between the "control autoclaved" pork group and the groups given autoclaved or irradiated pork.

Food intake

The food intake of the animals estimated in week 1, 2, 5, 9, 12, 20, 36, 52, 68, 84, 101 and 116 is given in table 7. It was clear that all animals on the pork diets had an increased food intake compared to the animals on the standard diet, but there were no differences between the "control autoclaved" pork group and the autoclaved or irradiated groups.

Behaviour, appearance and mortality

The behaviour and appearance of the rats remained normal during the first 68 weeks. Thereafter mortality was noticed in all groups, especially in the male rats (table 8). The survival curves for the rats are given in figure 3 for the female rats, and for the males in figure 4. Neither autoclavation nor irradiation had any obvious influence on mortality compared to controls.

Biochemistry

The results of the GPT, GOT, Alk.Pase, urea and glucose (104 weeks only) determinations in the serum, at week 26 and 104, are given in table 9 and 10 respectively.

After both periods the Alk.Pase activity in the serum of male rats on a standard diet was higher than that of the animals in the autoclaved or irradiated groups. For the females this was only found after 26 weeks. Furthermore, slight changes compared to the "control autoclaved" group were found incidentally.

Urinalysis only revealed a decreased protein concentration in the autoclaved group and the irradiated controls. No other changes were noted (table 11, 12, 13).

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Haematology

During the entire experimental period no consistent alterations in red blood cell parameters were noticed (table 14 to 23).

In male rats lymphocytosis or a tendency to lymphocytosis was found after 13 (table 15) and 52 (table 19) weeks, respectively. At the termination of the experiment no indication for lymphocytosis was present (table 23).

In the female rats leucocytopenia in group 3, 4 and 5 (table 18) was detected after 52 weeks.

At the end of the experiment no treatment related differences were found in the number and distribution of red and white cells (table 22 and 23).

Organ weights

The results of the organ weight determinations for female and male rats are given in table 24 and 25 respectively. The absolute and relative weight of the thyroid of male and female animals of the standard diet group was significantly lower compared to group 2. The pituitary weight in the females of group 3 was increased compared to group 2. The absolute liver weight in the males of group 3 and 6 and the relative weight of the liver in the males in group 3 was significantly increased.

Pathology

Macroscopic examination of the rats killed at termination of the experiment as well as the intercurrently autopsied animals that died or were killed because of ill health, did not suggest that any gross changes were induced by the dietary regimen. Lung and kidney changes were relatively rare, only some cases of pneumonia and glomerulonephrosis were seen. In most of the livers small foci were present consisting of aggregates of inflammatory cells sometimes accompanied by single cell necrosis. In a number of cases Bacillus piliformis could be detected in these foci. Moreover, larger, clearly allineated foci were seen especially in female animals. These foci consisted of cells with large vacuoles and a small pycnotic nucleus. Those cells contained both PAS positive, diastase resistant material and neutral fat as was demonstrated with the Oil Red O method. In many foci variable amounts of inflammatory, mainly mononuclear cells were seen, sometimes associated with fibrocytic activity. These foci were seen in all groups and their etiology is unknown. The number of animals showing hyperplastic changes in the urinary bladder was relatively high in males of group 3 and 4. These changes were, however, also seen in control rats and were not considered to be related to the treatment.

Table 26 presents the non-neoplastic histopathological changes observed in 10 female and 10 male animals that survived the experimental period. The observed pathological lesions were about equally present in the various groups or occurred in a single animal.

Incidence, site and type of tumours in rats that died or were killed in extremis or at the end of the experiment are presented in tabel 27. Common neoplasms in both sexes were chromophobe tumours of the pituitary, benign phaeochromocytomas and cortical adenomas of the adrenals and mammary fibroadenomas in female rats.

Whether treatment resulted in an earlier appearance of tumours was also analysed. Table 28 lists the tumour incidence of the various groups in chronological order. From these data no relevant differences in tumour incidence at the various periods between the experimental groups can be observed.

Discussion

From the results of all tested parameters in this relay study no adverse effects (food intake, growth, mortality, haematology, biochemistry, organ weights, pathology, tumor incidence) attributable to the irradiation used could be established. A decrease in the weight of the thyroid in males and females was detected in the "standard" group compared to the "control autoclaved".

The level of irradiation nor the amount of nitrite did alter the total concentrations of nitrosamines in pork samples, which were generally quite low.

All 5 experimental groups with a 35% pork diet showed a significant higher food intake than the group with standard rat feed. The higher food intake of the "pork" groups can be explained by the higher water content of these diets. This effect was comparable in the males and females. Between the 5 pork groups there was no difference.

All 5 experimental groups with a 35% pork diet showed a higher body weight compared to the group with standard rat feed. This effect was more pronounced in the females than in the males. Between the 5 experimental groups no differences were detected. This means that the high pork content of the diet and not the process of irradiation caused the increase in body weight. This is in contrast with the slight depression in body weight gain in mice and dogs which were given irradiated bacon or pork diets (9). In an earlier experiment the weight gain of female rats (but not in the males) on irradiated conventional (e.g. not pork supplemented) diet was significantly lowered (1). The general condition, food intake and body weight gain of pigs fed an irradiated diet did not differ from the control pigs (2). In conclusion, long term feeding of irradiated diets showed no influence on body weight gain.

Neither autoclavation nor irradiation had any effect on behaviour, appearance or mortality of the rats during the whole experimental period. These findings are in contrast with a reported increase in mortality in rats fed irradiated bacon or pork meat (9).

During the whole experimental period no alterations in erythrocytes were found. This is in agreement with an earlier study in rats (1) but not with reduced red blood cell counts in dogs and rats (9). Although not pathologically confirmed, the possibility exists that the alterations in the white blood picture found at intercurrent intervals in different experimental groups are not treatment or time related but due to infections. Van Logten et al. (1) also found a shift from lymphocytes towards neutrophilic cells.

In most cases the Alk.Pase activity in the serum of rats on a standard diet is higher than that of the autoclaved or irradiated groups. However, no differences were found between the autoclaved control and the other treatment groups. Moreover, neither liver weight nor the histopathology of the liver or intestines were different from the controls. Therefore it can be concluded that irradiation of the diet did not affect the liver or the intestines of the rats, but that the lower Alk.Pase activity of all treatment groups is due to the addition of meat to the diet. The results of this study are in agreement with the findings related to possible liver effects obtained in an earlier experiment (1).

Van Logten et al. (1) did suggest a possible influence of irradiated feed on kidneys of rats. In the present study according to urinalysis, weight of kidneys and histological evaluation of this organ no indication for kidney damage was found.

The non-neoplastic histopathological changes are common findings in ageing rats of this strain and were equally distributed amongst the various groups. Therefore these changes were not considered to be related to the treatment.

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The animals had no tumours of any particular or unusual type; all tumours listed are common in the strain of rats used (8). Although in a few cases marked differences in tumour incidences were seen between the various groups, these differences were not considered to be related to the feeding of the various diets (e.g. total number of primary tumours group 3, females; parafollicular cell adenoma in thyroid group 3, females; benign phaeochromocytomas in group 5, females; cortical adenomas in adrenals group 6, males; mammary fibro adenomas in females, group 2).

From the data on tumour appearance and incidence of all the groups (including controls) it was concluded that the latency period was comparable between the various groups. These results do not confirm indications from another study reporting a higher incidence in the development of tumours in animals on irradiated diets (9).

Renner et al. (10) found no evidence of mutagenic activity caused by irradiation of meat (e.g. chicken) detected by means of short term test systems. The absence of mutagenic potential of irradiated meat is in agreement with the absence of a higher tumour incidence in this report.

In conclusion:

From the results of this relay study no adverse effect attributable to the irradiation used could be established.

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Table 1. Composition of the curing brine (%)

	50 ppm $NaNO_2$	200 ppm $NaNO_2$
sodium chloride	24	24
polyphosphate (Curafos)	3.3	3.3
sodium ascorbate	0.55	0.55
sodium glutamate	0.2	0.2
glucose	4.4	4.4
sodium nitrite	0.055	0.22
water	67.5	67.33

Table 2. Chemical analysis of irradiated or autoclaved pork

parameter	group number			
	2	3	4	5 + 6
moisture (%)	74.9 ± 0.2 (6)	74.1 ± 0.8 (8)	74.6 ± 0.7 (6)	73.7 ± 0.7 (11)
protein (%)	18.8 ± 0.5 (6)	17.8 ± 0.4 (8)	19.4 ± 0.7 (6)	18.9 ± 0.4 (11)
moisture/protein	4.0 ± 0.1 (6)	4.2 ± 0.1 (8)	3.9 ± 0.2 (6)	3.9 ± 0.1 (11)
fat (%)	2.1 ± 0 (2)	4.4 ± 1.0 (8)	2.2 ± 0.4 (2)	3.0 ± 0.1 (4)
salt (NaCl) (%)	2.2 ± 0.1 (6)	2.3 ± 0.1 (8)	2.2 ± 0.1 (6)	2.3 ± 0.1 (11)
nitrite (ppm $NaNO_2$)	5.3 ± 2.3 (6)	6.5 ± 1.6 (8)	11.8 ± 2.1 (6)	13.1 ± 2.7 (11)
P_2O_5 (%)	0.67 ± 0.01 (6)	0.65 ± 0.02 (8)	0.67 ± 0.02 (6)	0.69 ± 0.01 (11)
pH	6.0 ± 0.4 (6)	6.2 ± 0.1 (8)	6.2 ± 0.1 (6)	6.1 ± 0.1 (11)
jelly (%)	10.5 ± 6.3 (6)	11.7 ± 1.5 (8)	1.9 ± 1.7 (6)	2.7 ± 1.3 (8)

Between brackets number of analysis

Table 3. Concentration of 5 volatile N-nitrosamines (µg/kg) in 3 samples pork

group number	nitrosamine					
	NDMA	NDEA	NDBA	NPYR	NPIP	total
1	0.2	-	-	-	-	0.2
2	n.d.	0.1	n.d.	0.3	n.d.	0.4
5	n.d.	0.3	n.d.	n.d.	n.d.	0.3
6	n.d.	0.4	n.d.	n.d.	n.d.	0.4

n.d.: not detectable (< 0.1 µg/kg)

- : not analyzed

NDMA: N-nitrosodimethylamine

NDEA: N-nitrosodiethylamine

NDBA: N-nitrosodi-n-butylamine

NPYR: N-nitrosopyrrolidine

NPIP: N-nitrosopiperidine

Table 4. Composition (%) of the standard and supplemented diet

.compound	standard diet (Muracon SSP-Tox)	supplemented diet
casein	18	8.9
soya protein	4.5	5.2
maize meal	22.4	26.0
dextropharm/glucose	27.3	31.1
akufloc/cellulose	10.4	11.9
soya bean oil	4.5	5.2
fat	2.3	0.7
calcium diphosphate	3.2	3.9
potassium carbonate	-	0.915
premix (containing vitamines and trace elements)	2.3	2.6
methionine	0.09	0.10
choline chloride (50 %)	0.29	0.33
potassium chloride	0.909	0.054
sodium chloride	0.364	-
sodium carbonate	0.455	-
ketjensil (free flowing agens)	2.7	3.1
butylated hydroxytoluene (BHT)	0.009	0.009

Table 5. Analysis of the experimental diets containing 35 % autoclaved or irradiated pork at 2 and 9 months after the start of the experiment

parameter (%)	group number					
	1	2	3	4	5	6
<u>after 2 months</u>						
protein	19.2	14.8	14.2	14.8	14.8	15.1
fat (ether extract)	7.3	6.1	6.1	5.9	6.6	5.9
cellulose	8.2	6.8	6.9	6.7	7.0	6.0
moisture (80°C vacuo)	7.8	33.5	34.0	36.0	32.5	34.0
chloride	0.8	0.6	0.6	0.6	0.6	0.7
<u>after 9 months</u>						
protein	19.6	15.6	14.7	14.9	15.1	14.5
fat (ether extract)	6.9	5.0	5.2	4.7	5.1	5.5
cellulose	7.8	6.3	6.1	6.1	6.1	5.6
moisture (80°C vacuo)	5.2	30.6	29.9	32.0	30.5	31.5
chloride	0.8	0.5	0.8	0.6	0.6	0.6

Table 6. Average initial body weight (g) and weight gain (g) of rats fed 35% autoclaved or irradiated pork

Group	number of rats	initial body weight	weight gain after (weeks)							
			6	12	36	52	68	84	100	116
females										
1	50	114	71***	97* (49)	137*** (48)	144*** (47)	175 (46)	187*** (42)	191 (38)	173 (32)
2	50	114	80	106	162	179 (49)	191 (49)	228 (45)	192 (40)	184 (31)
3	49	110	77	103	162	172	199	228 (41)	196 (39)	183 (30)
4	42	111	79	107	169	187	211 (41)	245 (35)	202 (31)	189 (23)
5	50	109	78	101	163 (48)	180 (48)	209 (47)	238 (44)	209 (40)	205 (33)
6	50	112	83	111	178* (47)	197* (47)	215 (45)	254* (44)	215 (39)	204 (26)
males										
1	50	135	161	219	296* (49)	306** (48)	342* (43)	361* (41)	337 (30)	289 (23)
2	50	132	155	220	323 (49)	347 (48)	374 (45)	398 (41)	339 (32)	297 (20)
3	50	134	145**	211 (49)	323 (48)	344 (48)	360 (46)	398 (38)	330 (29)	274 (21)
4	50	140	157	229	339	356	389 (48)	419 (45)	350 (38)	327 (20)
5	50	128	150	211	314 (48)	343 (47)	383 (45)	427 (38)	357 (33)	339* (21)
6	50	131	156	220	335	365 (47)	399 (46)	432 (40)	361 (34)	323 (23)

( ) number of rats still alive

* significant different from group 2: $0.01 \leq P < 0.05$

** significant different from group 2: $0.001 \leq P < 0.01$

*** significant different from group 2: $P < 0.001$

Table 7. Average food intake (g/animal/day) of 20 female and 20 male rats fed 35 % autoclaved or irradiated pork

group	week 1	week 2	week 5	week 9	week 12	week 20	week 36	week 52	week 68	week 84	week 101	week 116
females												
1	11 ***	12 ***	12 ***	11 ***(19)	11 ***(19)	11 ***(18)	11 ***(18)	11 ***(17)	11 ***(17)	11 ***(16)	12 ***(15)	13 ***(13)
2	16	18	16	15	16	17	17	17 (19)	19 (19)	22 (17)	25 (13)	27 (11)
3	16	18	16	15	16	17	16	17	19	20 (15)	22 (15)	25 (11)
4	15	17	16	16	16	17	16	18	20	22 (18)	23 (16)	26 (11)
5	16	18	16	15	15	16	17 (19)	18 (19)	20 (18)	20 (17)	23 (14)	24 (13)
6	15	17	16	16	17	17	17 (19)	19 (19)	21 (19)	18 * (19)	24 (18)	26 (11)
males												
1	15**	18***	19***	19***	18***	17***	15***	14 ***(19)	16 ***(16)	14 ***(14)	14 ***(9)	17 ***(8)
2	17	24	24	23	23	22	22	22 (19)	26 (18)	28 (16)	26 (11)	30 (6)
3	17	25	25	24	24	24	23	23	25	24 (12)	23 (8)	20 (6)
4	17	25	25	25*	25	24	24	24	27 (19)	24 (18)	27 (14)	29 (9)
5	17	24	24	23	22	22	21	22	24 (19)	24 (15)	24 (14)	28 (8)
6	17	23	24	23	23	23	21	22 (18)	25 (18)	23 (14)	24 (11)	27 (10)

( ) number of animals deviating from 20

* 0.01 ≤ P < 0.05
** 0.001 ≤ P < 0.05
*** P < 0.001

Table 8. Cumulative mortality for rats fed 35 % autoclaved or irradiated pork during 125 weeks

group	females/ males	initial number of rats	mortality at week														
			12	20	27	36	44	52	60	68	76	84	92	100	108	116	125
1	females	50	1	2	2	2	2	3	4	4	6	8	10	12	14	18	23
	males	50	0	1	1	1	2	2	3	7	8	9	15	20	24	27	37
2	females	50	0	0	0	0	0	1	1	1	3	5	6	10	15	19	24
	males	50	0	1	1	1	1	2	3	5	6	9	12	18	25	30	36
3	females	49	0	0	0	0	0	0	0	0	3	8	9	10	14	19	27
	males	50	1	1	1	2	2	2	2	4	8	12	16	21	25	29	37
4	females	42	0	0	0	0	0	0	0	1	3	7	8	11	16	19	21
	males	50	0	0	0	0	0	0	0	2	4	5	10	12	23	30	38
5	females	50	0	0	0	2	2	2	3	3	4	6	8	10	12	17	24
	males	50	0	1	2	2	2	3	4	5	10	12	14	17	25	29	35
6	females	50	0	0	1	3	3	3	4	5	6	6	8	11	17	24	29
	males	50	0	0	0	0	0	3	3	4	5	10	12	16	21	27	31

Table 9. Bio-chemical determinations in serum of rats fed 35 % autoclaved or irradiated pork after 26 weeks. Values are for groups of 5 female and 5 male animals (means ± s.d.)

group	parameter			
	SGPT (u/l)	SGOT (u/l)	Alk.Pase (u/l)	urea (mmol/l)
females				
1	18 ± 10	90 ± 17	79 ± 11**	6.6 ± 0.4
2	17 ± 9	81 ± 17	53 ± 6	6.8 ± 0.8
3	18 ± 6	79 ± 12	57 ± 10	7.3 ± 1.2
4	20 ± 6	81 ± 10	67 ± 10*	7.5 ± 0.9
5	28 ± 18	86 ± 16	52 ± 8	8.0 ± 1.0
6	14 ± 4	71 ± 11	58 ± 18	7.1 ± 1.6
males				
1	15 ± 3	85 ± 13	89 ± 11*	6.3 ± 1.1
2	16 ± 5	74 ± 14	68 ± 13	6.3 ± 0.8
3	13 ± 1	65 ± 7	74 ± 19	7.7 ± 4.9
4	11 ± 2	66 ± 5	59 ± 9	6.2 ± 0.7
5	17 ± 3	83 ± 9	74 ± 3	6.4 ± 0.6
6	11 ± 1	68 ± 12	75 ± 10	7.0 ± 0.9

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 10. Bio-chemical determinations in serum of rats fed 35 % autoclaved or irradiated pork after 104 weeks. Values are for groups of 5 female and 5 male animals (means ± s.d.)

group	parameter				
	SGPT (u/l)	SGOT (u/l)	Alk.Pase (u/l)	urea (mmol/l)	glucose (mmol/l)
females					
1	24 ± 8	127 ± 31	81 ± 14	6.1 ± 0.8	6.4 ± 0.7
2	26 ± 6	125 ± 22	93 ± 33	6.2 ± 0.5	6.1 ± 0.3
3	25 ± 11	128 ± 50	77 ± 20(4)	6.2 ± 1.4	6.4 ± 0.4
4	25 ± 8	109 ± 23	79 ± 34	5.8 ± 0.6	6.3 ± 0.4
5	26 ± 4	136 ± 23	59 ± 10	6.2 ± 1.1	6.3 ± 1.0
6	22 ± 5	108 ± 17	76 ± 11	6.7 ± 0.9	6.5 ± 0.9
males					
1	21 ± 4	147 ± 28*	110 ± 17*	5.1 ± 0.6	5.9 ± 0.5
2	22 ± 6	107 ± 23	84 ± 10	5.6 ± 0.3	6.1 ± 1.0
3	20 ± 7	79 ± 13*	89 ± 26	5.1 ± 1.3	6.9 ± 0.4
4	26 ± 10	126 ± 44	83 ± 51	4.7 ± 0.5**	7.1 ± 1.1
5	21 ± 7	145 ± 49	92 ± 19	6.4 ± 1.7	6.5 ± 1.1
6	23 ± 13	105 ± 43	97 ± 21	5.0 ± 0.5	6.8 ± 0.5

* 0.01 ≤ P < 0.05

** 0.001 ≤ P < 0.01

Table 11. Urinalysis of rats fed 35 % autoclaved or irradiated pork after 104 weeks. Values are for 8 female and 8 male animals (means ± s.d.)

	creatinine	protein	protein/creatinine
females			
group 1	2.1 ± 0.7	4.5 ± 0.8	2.4 ± 0.7
group 2	1.5 ± 0.6	3.5 ± 1.3	2.3 ± 0.4
group 3	1.7 ± 0.3	3.9 ± 0.9	2.4 ± 0.6
group 4	1.3 ± 0.6	3.2 ± 1.0	2.8 ± 1.2
group 5	2.0 ± 0.3	4.0 ± 0.7	2.0 ± 0.3
group 6	1.4 ± 0.3	2.9 ± 0.4	2.1 ± 0.3
males			
group 1	1.9 ± 0.8	5.1 ± 1.3	3.1 ± 1.3
group 2[x]	2.3 ± 1.0	4.8 ± 0.8	2.4 ± 0.9
group 3	2.2 ± 0.8	3.6 ± 0.7**	1.8 ± 0.3
group 4	1.8 ± 0.5	3.6 ± 1.2*	2.1 ± 0.8
group 5	2.8 ± 1.3	5.1 ± 2.0	1.9 ± 0.4
group 6	2.3 ± 0.8	4.5 ± 1.4	2.0 ± 0.7

x: means of 7

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 12. Semi-quantitative urinalysis of rats fed 35 % autoclaved or irradiated pork after 26 weeks

group	number of rats	pH			protein				glucose	keton bodies	bilirubin	blood		
		6	6½	7	S	Mo	Mu	V	neg	neg	neg	neg	S	Mo
females														
group 1	10	9	1		2	7	1		10	10	10	9		1
group 2	10	7	1	2	3	6	1		10	10	10	10		
group 3	10	7	2	1	3	7			10	10	10	10		
group 4	10	8	1	1	2	8			10	10	10	10		
group 5	10	9	1		2	5	2	1	10	10	10	8	1	1
group 6	10	8	2		2	7	1		10	10	10	10		
males														
group 1	10	9	1			5	5		10	10	10	10		
group 2	10	10				7	3		10	10	10	10		
group 3	10	6	4		1	7	2		10	10	10	10		
group 4	10	3	7		1	8	1		10	10	10	10		
group 5	10	8	2			7	3		10	10	10	10		
group 6	9	7	2			6	3		9	9	9	8	1	

neg = negative S = some Mo = moderate Mu = much V = very much

Table 13. Semi-quantitative urinalysis of rats fed 35 % autoclaved or irradiated pork after 104 weeks

group	number of rats	parameter																	
		pH					protein				glucose		keton bodies		bilirubin	blood			
		5	5½	6	6½	7	S	Mo	Mu	V	neg	S	neg	S	neg	neg	S	Mo	Mu
females																			
group 1	8			8				7	1		8		8		8	6	1	1	
group 2	8			6	1	1	2	2	4		7	1	8		8	5		3	
group 3	8		1	7			2	4	2		8		6	2	8	3	3	2	
group 4	8			8			1	7			8		8		8	3	1	3	1
group 5	8			8				6	2		8		8		8	4	2	2	
group 6	8	1		7			3	5			8		8		8	5	3		
males																			
group 1	8			8				2	4	2	6	2	7	1	8	1	3	4	
group 2	7			6	1			2	5		6	1	7		7	3	2	2	
group 3	8			8			1	7			8		6	2	8	5	1	2	
group 4	8			8			1	3	3	1	8		8		8	5	1	2	
group 5	8			8				3	5		8		8		8	7	1		
group 6	7			7			1	3	2	1	7		6	1	7	4	2		1

neg = negative S = some Mo = moderate Mu = much V = very much

Table 14. Haematological data of female rats fed 35 % autoclaved or irradiated pork after 13 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	9.8	9.5	9.6	9.6	9.6	9.6
haematocrit (l/l)	0.47	0.46	0.46	0.45	0.45	0.45
erythrocytes ($\times 10^{-12}$/l)	7.7	7.9	7.9	7.8	7.8	7.7
leucocytes ($\times 10^{-6}$/l)	13110	11260	12770	12110	12490	12270
MCV (fl)	60.6*	57.8	57.7	58.1	58.1	58.6
MCH (amol)	1279**	1208	1211	1226	1240	1239
MCHC (mmol/l)	21.1	20.9	21.0	21.1	21.4**	21.2
differential leucocyte count (abs.) ($\times 10^{-6}$/l)						
eosinophils	169	144	318*	133	154	131
basophils	13	18	29	17	19	5
neutrophils	1020	899	811	1331	1476	1130
lymphocytes	11309	9853	11091	10185	10408	10535
monocytes	600**	346	521*	443	434	470

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 15. Haematological data of male rats fed 35 % autoclaved or irradiated pork after 13 weeks

parameter	group 1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	10.5	10.1	10.2	10.3	10.1	10.1
haematocrit (l/l)	0.51**	0.48	0.49	0.49	0.48	0.48
erythrocytes ($\times 10^{-12}$/l)	8.7	8.7	8.5	8.4*	8.5	8.5
leucocytes ($\times 10^{-6}$/l)	15370*	11630	14220*	14400*	16160*	13340
MCV (fl)	58.1**	54.8	57.5*	57.9*	55.9	56.0
MCH (amol)	1210	1165	1205	1231*	1183	1188
MCHC (mmol/l)	20.8**	21.3	21.0	21.3	21.2	21.2
differential leucocyte count (abs.) ($\times 10^{-6}$/l)						
eosinophils	96	189	205	89	192	188
basophils	0	11	11	40	21	0
neutrophils	1176	902	906	779	1212	671
lymphocytes	13423*	10060	12562*	12939**	14166*	12078*
monocytes	674	468	535	554	570	403

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 16. Haematological data of female rats fed 35 % autoclaved or irradiated pork after 26 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	9.5	9.3	9.5	9.5	9.4	9.3
haematocrit (l/l)	0.46	0.45	0.46	0.47	0.45	0.46
erythrocytes (x 10^{-12}/l)	7.6	7.8	7.8	8.0	7.6	7.6
leucocytes (x10^{-6}/l)	8960	8530	7850	9150	8720	8110
MCV (fl)	60.8	58.3	58.9	58.5	59.9	60.3*
MCH (amol)	1248	1204	1224	1191	1246*	1230
MCHC (mmol/l)	20.5	20.7	20.8	20.3	20.8	20.4
differential leucocyte count (abs.) (x 10^{-6}/l)						
eosinophils	85	76	78	94	66	57
basophils	25	6	10	19	14	4
neutrophils	652	791	640	842	1174	837
lymphocytes	7803	7287	6780	7839	7088	6907
monocytes	395	371	343	355	378	305

* $0.01 \leq P < 0.05$

Table 17. Haematological data of male rats fed 35 % autoclaved or irradiated pork after 26 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	9.9	9.8	9.8	10.1	9.8	9.6*
haematocrit (l/l)	0.48	0.47	0.47	0.48	0.47	0.47
erythrocytes (x 10^{-12}/l)	8.3*	8.5	8.3*	8.4	8.4	8.3
leucocytes (x 10^{-6}/l)	11590	9350	9790	9900	11240*	9750
MCV (fl)	57.9**	55.4	57.2*	57.6*	56.2	55.9
MCH (amol)	1200**	1153	1189	1204*	1170	1149
MCHC (mmol/l)	20.7	20.8	20.8	20.9	20.8	20.6
differential leucocyte count (abs.) (x 10^{-6}/l)						
eosinophils	122	67	139	189*	209**	67
basophils	0	15	4	29	0	30
neutrophils	1064	946	1064	677	1169	661
lymphocytes	9950*	7928	8227	8600	9449	8681
monocytes	454	395	356	405	409	312

* 0.01 ≤ P < 0.05
** 0.001 ≤ P < 0.001

Table 18. Haematological data of female rats fed 35 % autoclaved or irradiated pork after 52 weeks

parameter	group 1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	9.8**	9.3	9.4	9.6	9.4	9.3
haematocrit (l/l)	0.46**	0.44	0.44	0.46*	0.44	0.44
erythrocytes (x 10^{-12})	7.8	7.6	7.5	7.7	7.6	7.5
leucocytes (x 10^{-6}/l)	11690	12870	9780*	10290*	9510**	11040
MCV (fl)	59.8	57.7	59.4	59.4	58.0	58.4
MCH (amol)	1264	1220	1260	1245	1236	1243
MCHC (mmol/l)	21.2	21.1	21.2	21.1	21.3	21.3
differential leucocyte count (abs.) (x 10-6/l)						
eosinophils	82	110	94	101	52	74
basophils	35	5	6	40***	11	6
neutrophils	1231	1855	1195	1036	691*	1112
lymphocytes	9860	10456	7999	8707	8383	9477
monocytes	482	444	486	406	373	371

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

*** $P < 0.001$

Table 19. Haematological data of male rats fed 35 % autoclaved or irradiated pork after 52 weeks

parameter	group 1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	9.7	9.8	9.9	10.1	9.8	9.8
haematocrit (l/l)	0.47	0.46	0.46	0.47	0.46	0.46
erythrocytes (x 10^{-12}/l)	8.1	8.2	7.9*	8.0	8.3	8.0
leucocytes (x 10^{-6}/l)	14270	12610	12680	12700	11930	13830
MCV (fl)	57.6*	55.6	58.6**	58.7**	55.3	56.8
MCH (amol)	1202	1184	1251**	1257**	1174	1216
MCHC (mmol/l)	20.9	21.3	21.4	21.5	21.2	21.4
differential leucocyte count (abs.) (x 10^{-6}/l)						
eosinophils	126	135	170	147	106	183
basophils	21	28	25	7	18	32
neutrophils	2691	2045	1297	947*	1029*	1471
lymphocytes	1 0590	9921	1 0662	111 39	10282	11557
monocytes	842	482	526	459	496	586

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 20. Haematological data of female rats fed 35 % autoclaved or irradiated pork after 104 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	9.1	8.5	7.6	8.0	8.5	8.4
haematocrit (l/l)	0.44	0.41	0.38	0.39	0.41	0.40
erythrocytes (x 10^{-12}/l)	7.2	6.9	6.2	6.4	6.8	7.1
leucocytes (x 10^{-6}/l)	12670	11680	13510	12950	10540	12760
MCV (fl)	60.7	58.8	62.3*	62.3*	60.5	58.2
MCH (amol)	1279	1223	1232	1237	1248	1216
MCHC (mmol/l)	20.9	20.8	19.9	20.0	20.8	20.9
differential leucocyte count (abs.) (x 10^{-6}/l)						
eosinophils	121	105	75	65	64	78
basophils	29	7	44	27	10	28
neutrophils	3038	3342	4733	4546	3153	3633
lymphocytes	8690*	7328	7782	7565	6785	8136
monocytes	792	898	876	746	527*	886

* $0.01 \leq P < 0.05$

Table 21. Haematological data of male rats fed 35 % autoclaved or irradiated pork after 104 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	10	10	10	10	10	10
haemoglobin (mmol/l)	8.9	9.2	8.9	9.3	8.9	8.7
haematocrit (l/l)	0.43	0.45	0.42	0.45	0.43	0.42
erythrocytes (x 10^{-12}/l)	7.2	7.9	7.4	7.5	7.5	7.4
leucocytes (x 10^{-6}/l)	16080	16000	13360	15090	15080	15570
MCV (fl)	58.8	56.3	56.9	59.9	57.2	56.6
MCH (amol)	1238	1169	1191	1233	1195	1170
MCHC (mmol/l)	20.9	20.7	21.0	20.6	20.8	20.6
differential leucocyte count (abs.) (x 10^{-6}/l)						
eosinophils	87	163	162	234	280	288
basophils	31	28	24	38	23	21
neutrophils	2643	4743	3342	4058	4260	3904
lymphocytes	11246	9833	8795	9614	9585	10290
monocytes	1073	1233	1038	1147	933	1068

Table 22 Haematological data of female rats fed 35 % autoclaved or irradiated pork after 125 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	23	26	20	20	20	21
haemoglobin (mmol/l)	8.1	8.7	8.1	8.4	8.7	8.5
haematocrit (l/l)	0.39	0.41	0.39	0.40	0.41	0.41
erythrocytes ($\times 10^{-12}$/l)	6.5	7.0	6.2*	6.7	6.9	6.6
leucocytes ($\times 10^{-6}$/l)	12043	8924	10170	10855*	11900	9567
MCV (fl)	63.9	58.6	63.2*	60.8	63.2*	62.6**
MCH (amol)	1319*	1233	1309*	1270	1334*	1290*
MCHC (mmol/l)	20.9	21.0	20.8	21.0	21.1	20.7
differential leucocyte count (abs.) ($\times 10^{-6}$/l)						
eosinophils	144	60	40	90	37	45
basophils	7	11	9	11	33	4
neutrophils	4154	2783	4181*	4343*	4004	3526
lymphocytes	6750	5216	5029	5686	6745	5214
monocytes	989	853	911	755	1081	777

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 23. Haematological data of the male rats fed 35 % autoclaved or irradiated pork after 125 weeks

parameter	group					
	1	2	3	4	5	6
number of rats examined	13	14	13	12	19	14
haemoglobin (mmol/l)	8.7	9.5	9.6	9.6	9.1	9.3
haematocrit (l/l)	0.42	0.45	0.46	0.46	0.43	0.44
erythrocytes (x 10^{-12}/l)	7.2	7.6	8.1	8.0	7.5	8.0
leucocytes (x 10^{-6}/l)	13931	11186	11154	13358	13953	14664*
MCV (fl)	59.9	59.2	56.8	57.6	57.7	55.8***
MCH (amol)	1226	1242	1196	1197*	1200	1173**
MCHC (mmol/l)	20.8	21.0	21.1	20.8	20.8	21.0
differential leucocyte count (abs.) (x 10^{-6}/l)						
eosinophils	101	152	114	84	90	141
basophils	49	22	22	22	39	40
neutrophils	5493	3340	3501	4915	5370	5485
lymphocytes	6869	6685	6443	7244	7228	7276
monocytes	1418	987	1075	1095	1226	1722

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

*** $P < 0.001$

Table 24. Absolute and relative organ weight in percentage to the body weight of female rats fed 35 % autoclaved or irradiated pork

organ	group 1	group 2	group 3	group 4	group 5	group 6
number of rats	22	26	19	20	21	19
body weight	286	303	301	290	306	298
heart	1.389	1.414	1.511	1.460	1.507	1.466
brain	2.00	2.02	2.03	1.97 *	1.97	1.99
liver	11.50	12.10	13.38 (18)	12.79	13.68	12.17 (18)
kidneys	3.07	3.18	3.25 (18)	3.10	3.27	3.10 (18)
spleen	0.769 (20)	0.894 (25)	0.960 (18)	0.887	0.988	0.869 (17)
adrenals	0.074	0.088 (25)	0.089 (18)	0.080	0.092 (20)	0.083 (18)
thyroid	0.035 **	0.049 (25)	0.056 (18)	0.051	0.049	0.051 (18)
pituitary	0.025 (19)	0.029 (24)	0.048 (18)	0.031 (17)	0.029 (18)	0.033
uterus	0.599	0.590 (24)	0.616 (18)	0.579	0.611	0.546
ovaries	0.054 (21)	0.063	0.059	0.061 (18)	0.062	0.064
coecum	0.911	0.973	0.948	0.988	0.962	0.947
relative weight (%)						
heart	0.494	0.473	0.512	0.507	0.602	0.491
brain	0.72	0.68	0.69	0.69	0.66	0.68
liver	4.05	4.02	4.45 (18)	4.43	4.51	4.09 (18)
kidneys	1.09	1.05	1.10 (18)	1.08	1.09	1.05 (18)
spleen	0.276 (20)	0.299 (25)	0.321 (18)	0.307	0.326	0.296 (17)
adrenals	0.0267	0.029 (25)	0.030 (18)	0.028	0.031 (20)	0.028 (18)
thyroid	0.013 *	0.016 (25)	0.019 (18)	0.018	0.016	0.017 (18)
pituitary	0.009 (19)	0.009 (24)	0.016 * (18)	0.011 (17)	0.009 (18)	0.011
uterus	0.211	0.197 (24)	0.214 (18)	0.207	0.203	0.184
ovaries	0.019 (21)	0.021	0.020	0.021 (18)	0.020	0.021
coecum	0.326	0.329	0.319	0.350	0.323	0.322

( ) divergent number of rats

* $0.01 \leq P < 0.05$

** $0.001 \leq P < 0.01$

Table 25. Absolute and relative organ weight in percentage to the body weight of male rats fed 35 % autoclaved or irradiated pork

organ	group 1	group 2	group 3	group 4	group 5	group 6
number of rats	13	14	14	12	14	19
body weight	406	405	404	430	401	432
heart	1.708	1.568	1.648	1.666	1.664	1.737
brain	2.15	2.08	2.13	2.17	2.12	2.13
liver	15.15	14.14	16.60**	16.18	14.06	15.94*
kidneys	4.09	3.98	4.12	4.07	3.62*	4.12
spleen	1.072** (12)	0.807	0.951* (13)	1.020 (11)	0.939 (12)	1.063
adrenals	0.092 (10)	0.091 (13)	0.129	0.075 (11)	0.089 (12)	0.115 (16)
thyroid	0.039*	0.049 (12)	0.061 (13)	0.065	0.059	0.062
pituitary	0.028 (11)	0.025 (12)	0.043 (12)	0.024 (11)	0.043 (13)	0.084
testes	2.288	2.669	2.627	2.374	2.460	2.454
prostate	0.210	0.198	0.184 (13)	0.179	0.185	0.242
coecum	1.272	1.237	1.246	1.255 (11)	1.130	1.237
relative weight (%)						
heart	0.426	0.397	0.421	0.400	0.425	0.421
brain	0.54	0.53	0.54	0.53	0.55	0.51
liver	3.76	3.56	4.20*	3.89	3.50	3.78
kidneys	1.02	1.01	1.05	0.97	0.93	0.99
spleen	0.267** (12)	0.202	0.242* (13)	0.245 (11)	0.233 (12)	0.262
adrenals	0.024 (10)	0.023 (13)	0.032	0.018 (11)	0.022 (12)	0.029 (16)
thyroid	0.010*	0.013 (12)	0.016 (13)	0.016	0.015	0.015
pituitary	0.008 (11)	0.006 (12)	0.011 (12)	0.006 (11)	0.011 (13)	0.023
testes	0.559	0.675	0.659	0.542	0.626	0.573
prostate	0.053	0.048	0.047 (13)	0.042	0.049	0.057
coecum	0.317	0.311	0.314	0.298	0.288	0.267

( ) divergent number of rats

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

Table 26. Incidence of histopathological findings (without tumours) in rats fed 35 % autoclaved or irradiated pork meat

	group	1		2		3		4		5		6	
	number	10	10	10	10	10	10	10	10	10	10	10	10
	sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
BRAIN total number examined		10	10	10	10	10	10	10	10	10	10	10	10
dilated ventricles			1						1		2		
calcium deposits in cerebrum				1			1		1		1	1	1
myelin degeneration			1						1				
HEART total number examined		10	10	9	10	10	10	10	10	10	9	10	10
myocardial degeneration		3	4	6	4	5	3	3	3	4	3	1	5
myocardial fibrosis		1	2	4	2	2	2	2	3	4	3	1	4
cartilaginous metaplasia						1							
focal myocarditis			1		2	2		2	3	1			1
slight endocarditis							1						
endocardial disease									1				
LUNGS total number examined		10	10	10	10	9	10	10	10	10	10	9	10
focal pneumonia			1				1		1				
necrotising pneumonia											1		
proliferative pneumonia								1					
interstitial pneumonia			3		2	1		2		2		2	2
alveolar macrophages		2	2	1	2	2	1	2	4	2	1	1	2
hyperemia			4		1		3		2		1	1	2
hemorrhages												1	
LIVER total number examined		10	10	10	10	10	9	10	10	10	10	10	10
bile pigment in hepatocytes				1	1								
vacuolisation				3		1		1	1	4	1	2	1
degeneration with or without necrosis						1		1	1	2	1		
focal fatty changes				2	1		1	1		1			
focal necrosis		3	3	4	1		1			4	1	1	3
foci of inflammation		2		2	2	4	3	2	2	2	3	3	6
foci with inflammatory and fatty changes		4		5		6		8	1	8	1	8	3
hepatitis		1								2			
hemorrhage											2		
hyperplastic areas or nodules		2	2	3	1			1	1	3	4	4	2
hepatocellular hyperplasia			1									1	
oval cell type bile duct proliferation			1										
cystadenomatous bile duct proliferation					1							1	
slight increase of bile ducts		3	3	4	1	6	1	2	2	3	3	3	4
tubular bile duct proliferation		1	5	4	3	1	2	2	2	2	4	5	3
pericholangitis		3	1	6	2	1	1	2	2	3	4	2	2
cholangiofibrosis		4	1	6	2		1		1	1	3	3	2
SPLEEN total number examined		9	10	10	10	10	10	10	10	10	10	10	10
slight hemosiderosis		3	1	3	4	3	3	4	4	1	4	2	3
moderate hemosiderosis		1	1	1	1		6	2	1	3		2	1
severe hemosiderosis			2	1	2				2	1	3	1	3
slight extramedullary hemopoiesis			1	1	2	1	6	1	2		1		2
moderate extramedullary hemopoiesis		2		5	2	6		3	2	5	1	4	2
severe extramedullary hemopoiesis			1	1		2		1		3	1		2
slight lymphoid depletion		2	1			2	1	1	1		1		1
many immunoblasts in sinusoids			1	1				3	1	1	1	1	
hyperemia						1	1		1		1		1
hemorrhage												1	
diffuse foci of necrosis							1						1
angiomatous proliferation						1	1	1				2	

Table 26. (continued 1)

group	1		2		3		4		5		6	
number	10	10	10	10	10	10	10	10	10	10	10	10
sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
<u>KIDNEYS</u> total number examined	10	10	10	9	10	10	10	10	10	10	10	10
calcium deposits in cortex											1	
calcium deposits in juxtamedullary area	5		3		3		2		1		4	
calcium deposits in pyelum	1	2		1	2	3	1	3	3	1	1	2
hydronephrosis	1	1										
lipofuscin pigment in tubules	2		4	1	4	1	2	3	4	1	2	2
swollen proximal tubules	3	1	4	1		2	1	3	2		2	1
thickened basal membranes in glomeruli	7	5	8	6	4	5	5	5	4	5	7	5
slight to moderate glomerulonephrosis	1	4	1	2	3	2	1	1	1	2	1	3
severe glomerulonephrosis		1						1				
hemorrhage in distal medulla											1	
descending glomerulonephritis		1										
focal inflammation		1	1				1					
chronic pyelitis									1			
pyelonephritis									1			
hyperplasia of pyelumepithelium		2			1	4		2	4	2	2	1
<u>PITUITARY GLAND</u> total number examined	9	7	4	6	8	6	8	8	8	8	8	7
cysts				1		1	1	4				
focal vacuolisation	1											
focal calcium deposits							1					
hyperplastic areas		2		3	3	4	3	6	1			2
hyperplasia of pars intermedia	1							1				
<u>THYROID GLAND</u> total number examined	10	6	8	8	8	8	7	9	10	9	10	10
diffuse parafollicular cell proliferation		2		1				1	1	2	1	
focal nodular parafollicular cell proliferation		1						1	1	1	3	1
multiple nodular parafollicular cell proliferation						1			3	1		
hyperplasia of parathyroid gland	2	1	1	3		3	1	2	2	3	4	2
<u>PANCREAS</u> total number examined	10	10	10	10	10	10	9	10	10	10	10	10
ectopic liver tissue	1											
focal pancreatitis		1										
acinar cells completely empty			1									
adenomatous duct hyperplasia	1	1	2	1	2	1	2	3	2	1	1	
severe atrophy with duct hyperplasia						1		1				
hyperplasia exocrine tissue								2				
hyperplasia islets of Langerhans		1		1		1				1		
<u>ADRENALS</u> total number examined	10	9	10	9	10	9	10	9	10	10	10	9
cortical hyperemia and/or hemorrhage	1								2		1	
hemorrhage with thrombus									1			
vacuolisation cortex		1	1								1	
irregular zona glomerulosa	5	2	7	1	5	2	5	3	4	3	3	3
cortical hyperplasia with/without vacuolisation	3	5	6	3	3	3	2	3	7	2	2	2
<u>OVARIES</u> total number examined	10		10		10		10		10		10	
senile atrophy	6		7		8		5		8		6	
follicular cysts	1				1		2		1			
hyperplasia of interstitial cells	1						3		1		2	
in corpus luteum many cholesterol cristals and giant cells			1									
<u>TESTIS</u> total number examined		10		10		9		10		10		10
slight atrophy of seminiferous tubules												1
unilateral degenerative atrophy		2		3		2		1		4		2
bilateral degenerative atrophy		3		3		3		5		2		3
periarteritis nodosa						1						
angioblast proliferation in interstitium with edema, macrophages and mast cells						1						

Table 26. (continued 2)

	group	1		2		3		4		5		6	
	number	10	10	10	10	10	10	10	10	10	10	10	10
	sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
UTERUS total number examined		10		10		10		10		10		10	
hydrometra		1		3		1		1				1	
glandular hyperplasia				2				2					
oöcyte in myometrium		1											
hyaline degeneration of stroma		6		7		7		5		7		5	
purulent endometritis		1											
PROSTATE GLAND total number examined			8		9		8		9		10		9
slight prostatitis			1		1				1				2
necrotising prostatitis			2		1		2				2		2
chronic prostatitis							3		2		2		
URINARY BLADDER total number examined		10	38	10	32	10	37	10	40	10	31	10	36
no diagnosis because of autolysis			9		4		8		16		6		4
proteinaceous concrement			1		3		1		2		2		2
cystitis			5		2		3						
slight hyperplasia epithelium			2		1		2		1	2	1		1
moderate hyperplasia epithelium							5		9		1		1
severe hyperplasia epithelium			3				4		1		2		3
very severe hyperplasia epithelium									1				
papillomatous hyperplasia epithelium							1						
SALIVARY GLANDS number examined		10	10	9	10	10	10	10	9	10	9	9	10
vacuolated cells in submaxillaris			1		2	1					1		
local atrophy acini + inflammation				1		1	1				2	2	4
slight duct metaplasia		1	1	2	2		1	1		1		1	
LN MESENTERIALIS total number examined		9	8	6	10	7	10	9	10	9	10	9	9
many eosinophils in sinusoids				1		1		2					
many immunoblasts in sinusoids												1	
mononuclear phagocytes		3	1	2	7	6	4	6	6	4	6	6	7
proliferation of endothelium													1
fatty changes in paracortical areas									1				
STOMACH total number examined		10	10	10	10	10	10	10	10	10	10	10	10
eosinophilis or mononuclear cells in submucosa		1	2	1	3	1	1	1	1	2	1	2	2
hyperkeratotic cyst in cardia		1			1								
hyperplasia cardia epithelium				1					1	1	2	2	2
gastritis				1							1		
atrophy glandular cells in fundus									1				
cystic tubules in fundus		4	2	5	1	6	3	5	2	6	1	2	
ulceration, hemorrhages and inflammation												1	
INTESTINES number examined at 6 levels		10	10	10	10	10	10	10	10	10	10	10	10
cellular infiltrates in propria		1	1	2				1		2	2		
increase of mucus in ileum									1				
hyperplasia Peyers' patch				2	1								
local hyperplasia caecum epithelium							1		2				
necrotising colitis													1
ulcus in colon with peritonitis									1				
polyp small intestine							1						

Table 26. (continued, 3)

	group	1		2		3		4		5		6	
	number	10	10	10	10	10	10	10	10	10	10	10	10
	sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
SPINAL CORD total number examined		10	10	10	9	9	8	9	10	10	10	7	10
slight radiculoneuropathy		3	4	2	1	2	3	2	3	3	2	1	1
moderate radiculoneuropathy		2	1	2	2	1	1	3	2	4	3	1	3
severe radiculoneuropathy		2	2	1	1								
hemorrhage in one of the roots												1	
severe degeneration of neurons in grey tissue				1									
N.ISCHIADICUS total number examined		10	9	8	9	10	9	10	9	9	10	9	9
slight Wallerian degeneration with segmental demyelinisation			1	1		1		3	1			1	
moderate Wallerian degeneration with segmental demyelinisation		3		2	4	4	3	5	3	3	3	5	5
severe Wallerian degeneration with segmental demyelinisation		5	6	5	4	4	5	2	4	5	7	3	4
OTHER PATHOLOGICAL CHANGES													
chronic peritonitis abdomen												1	
polyarteritis nodosa										1		1	
necrotising dermatitis							1						
purulent dermatitis with abscess		1	1										
steatitis with abscess											1		

Table 27. Incidence, site and type of tumours in the different groups of rats (survivors and non-survivors together)

site and type of tumours	females						males					
group	1	2	3	4	5	6	1	2	3	4	5	6
initial number of rats	50	50	49	42	50	50	50	50	50	50	50	50
number of rats that survived 24 months	37	35	39	28	36	39	30	29	28	36	32	30
number of rats that survived 30 months	31	30	30	21	28	22	22	18	19	16	17	19
number of rats examined	48	50	49	42	50	49	50	50	50	49	50	48
number of tumour bearing rats	32	42	49	37	40	38	36	34	40	37	40	43
number of primary tumours	47	69	95	62	77	67	52	62	58	52	62	69
number of rats having one tumour	19	21	20	21	19	22	22	13	27	26	24	24
number of rats having two tumours	11	17	18	11	9	10	12	14	8	7	11	12
number of rats having three tumours	2	2	5	1	9	3	2	7	5	4	4	7
number of rats having four tumours	-	2	6	4	3	4	-	-	-	-	1	-
PITUITARY GLAND												
chromophobe adenoma	9	17	29	20	18	12	20	16	21	21	13	25
chromophobe adenoma with cellular pleomorphy	4	2	2	1	2	6	2	1	3	1	10	1
eosinophilic carcinoma	1		2			1					1	
pars neuralis tumour									1			1
THYROID												
parafollicular cell carcinoma	1	2	3	2	7	6		3	4	1	3	2
follicular adenoma			1		1					1		
parafollicular cell adenoma			8	4				1	2	1		
ADRENALS												
benign phaeochromocytoma	4	11	7	5	17	5	11	10	9	6	7	11
malign phaeochromocytoma	1	1	1			1	2	4	4	1	4	1
cortical adenoma	2	9	2	2	5	5	1	5	2	2	1	10
cortical carcinoma		1	2			1	2					
MAMMARY GLAND												
fibroadenoma	7	19	16	10	8	12	2	3		1		
adenoma	5		2	1	1	1						
adenocarcinoma	1		6	1	2	2	1					
cystadenoma					2							
papillary carcinoma				1		1						
SKIN AND SUBCUTIS												
fibroma		1	1	4	1	1	1	4		2	2	3
fibrosarcoma	2		1			2	1	1			2	
squamous cell carcinoma		1		1		1		1			1	2
lipoma			1		2	1		2	1			
basal cell carcinoma					1							
trichoepithelioma					1		1				1	1
sebaceous gland carcinoma					1			1			1	
squamous cell papilloma											1	
reticulum cell carcinoma											1	
ABDOMINAL CAVITY												
anaplastic carcinoma							1					
fibrosarcoma				1						1		
malign unclassified tumour										1		
fibroma			1									
reticulum cell carcinoma											1	
BRAIN												
granular cell myoblastoma									1	2	1	1
ependymoma								1			2	1
astrocytoma	1											
glioma		1								1		
mixed glioma										1		

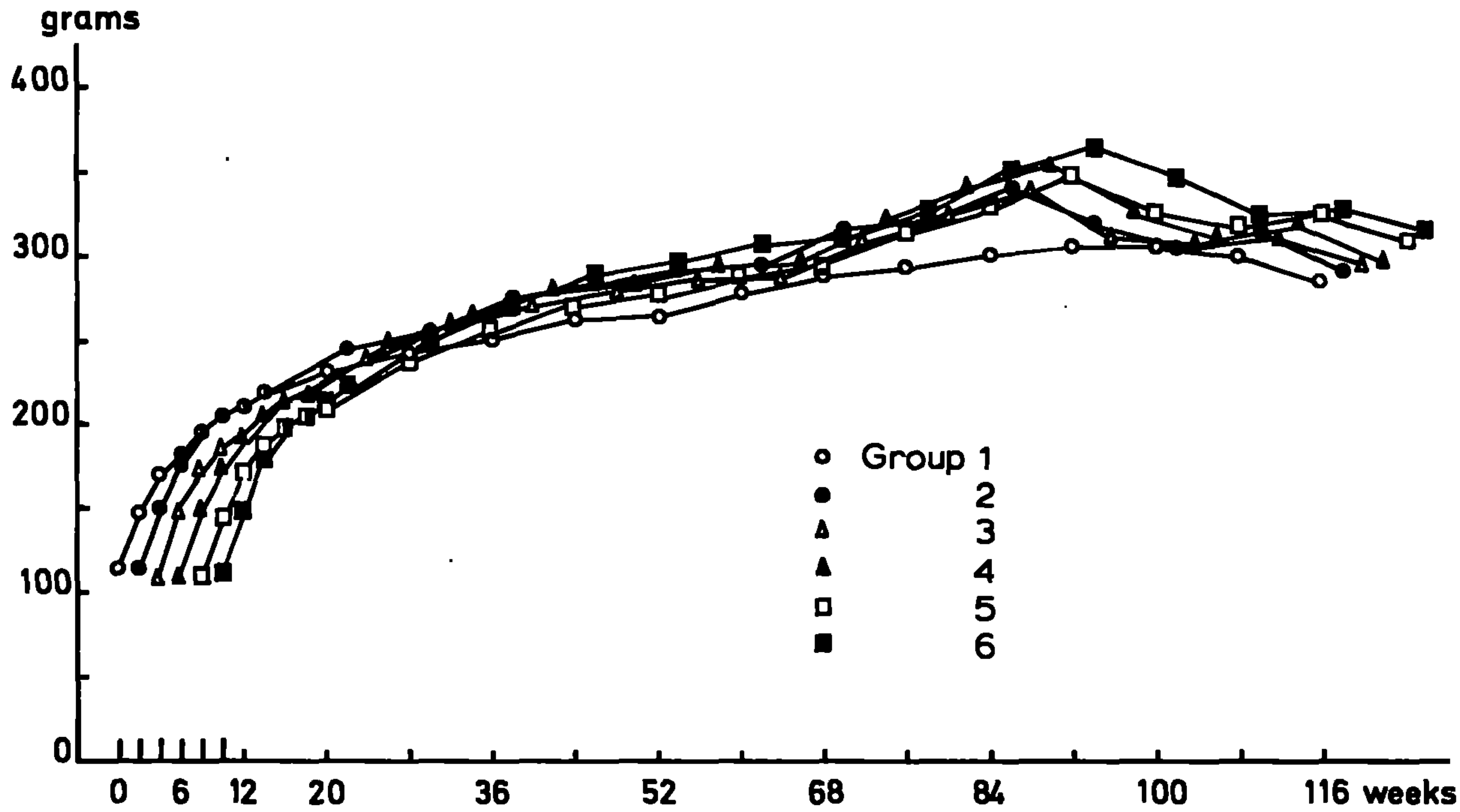
Table 27. (continued)

site and type of tumours	group	females 1	females 2	females 3	females 4	females 5	females 6	males 1	males 2	males 3	males 4	males 5	males 6
<u>SPLEEN</u>													
haemangio-endotheliosarcoma												1	
angiosarcoma												1	
reticulum cell sarcoma										1			
<u>COLON</u>													
papilloma										1	1		
<u>SMALL INTESTINES</u>													
papillary adenoma		1											
papillary adenocarcinoma		1						1					
fibrosarcoma					1			1		1	1		
<u>THYMUS</u>													
lymphoma							1						
lymphosarcoma			1										1
squamous cell carcinoma										1			
<u>PANCREAS</u>													
isletcell tumours		1		1		2		1	1	1		1	3
adenocarcinoma							1						
<u>LIVER</u>													
cholangiocystadenoma					1				1		1		
livercell carcinoma				2									
<u>URINARY BLADDER</u>													
papilloma										2			
<u>HAEMATO-LYMPHOPOIETIC SYSTEM</u>													
lymphocytic leukemia		5	2		2	3	3	2	4	1	3	3	1
lymphosarcoma									1				1
reticulum cell sarcoma						1							
haemangio-endotheliosarcoma												1	
lymphoma					1								
<u>LEGS</u>													
fibrosarcoma				1					1				1
osteosarcoma						1				1			
<u>PLEURAL CAVITY</u>													
angiosarcoma												1	
<u>UTERUS</u>													
fibromatous polyp				2	2								
fibrosarcoma				1									
cystadenoma			1										
leiomyoma				1			1						
<u>OVARIES</u>													
granulosa cell tumour		1			2								
lipoma						1							
<u>TESTES</u>													
seminoma								1					
<u>KIDNEYS</u>													
clearcell tumour								1					
tubular adenoma									1	1			
adenocarcinoma				2							1	1	
papilloma							1						
angiosarcoma													1
<u>STOMACH</u>													
adenocarcinoma						1							
fibrosarcoma											1		
<u>HEART</u>													
mesothelioma							1						
<u>SPINAL COLUMN</u>													
osteosarcoma											1	1	2

Table 28. Chronology of tumour incidence (number of tumour bearing rats/number of rats examined) during the following periods (months)

group	sex	0-12	12-14	14-16	16-18	18-20	20-22	22-24	24-26	26-28	28-30
1	♀	0/1	0/0	0/1	2/2	2/2	1/3	0/2	1/1	3/5	23/31
	♂	0/2	0/0	3/5	1/1	2/3	5/5	4/4	4/4	3/4	14/22
2	♀	0/0	2/2	0/0	2/3	2/2	0/1	6/7	3/3	1/2	26/30
	♂	0/2	1/2	0/1	2/3	2/4	2/2	4/7	8/9	2/2	13/18
3	♀	0/0	0/0	0/0	3/3	5/5	1/1	2/2	5/5	4/4	29/30
	♂	0/2	0/0	0/0	4/6	3/5	4/4	5/5	7/7	2/2	15/19
4	♀	0/0	0/0	1/1	2/3	3/4	1/1	4/4	4/4	3/3	18/21
	♂	0/0	0/0	1/2	0/2	1/1	3/4	2/4	10/12	7/7	12/16
5	♀	0/2	1/1	1/1	3/3	2/3	1/3	1/1	3/4	3/4	26/28
	♂	2/3	0/0	1/2	3/5	1/3	1/1	4/4	6/8	7/7	16/17
6	♀	1/3	1/1	1/1	0/0	0/1	1/1	2/3	9/11	5/6	19/22
	♂	2/2	0/1	2/2	2/2	2/5	3/3	3/4	5/5	6/6	18/19

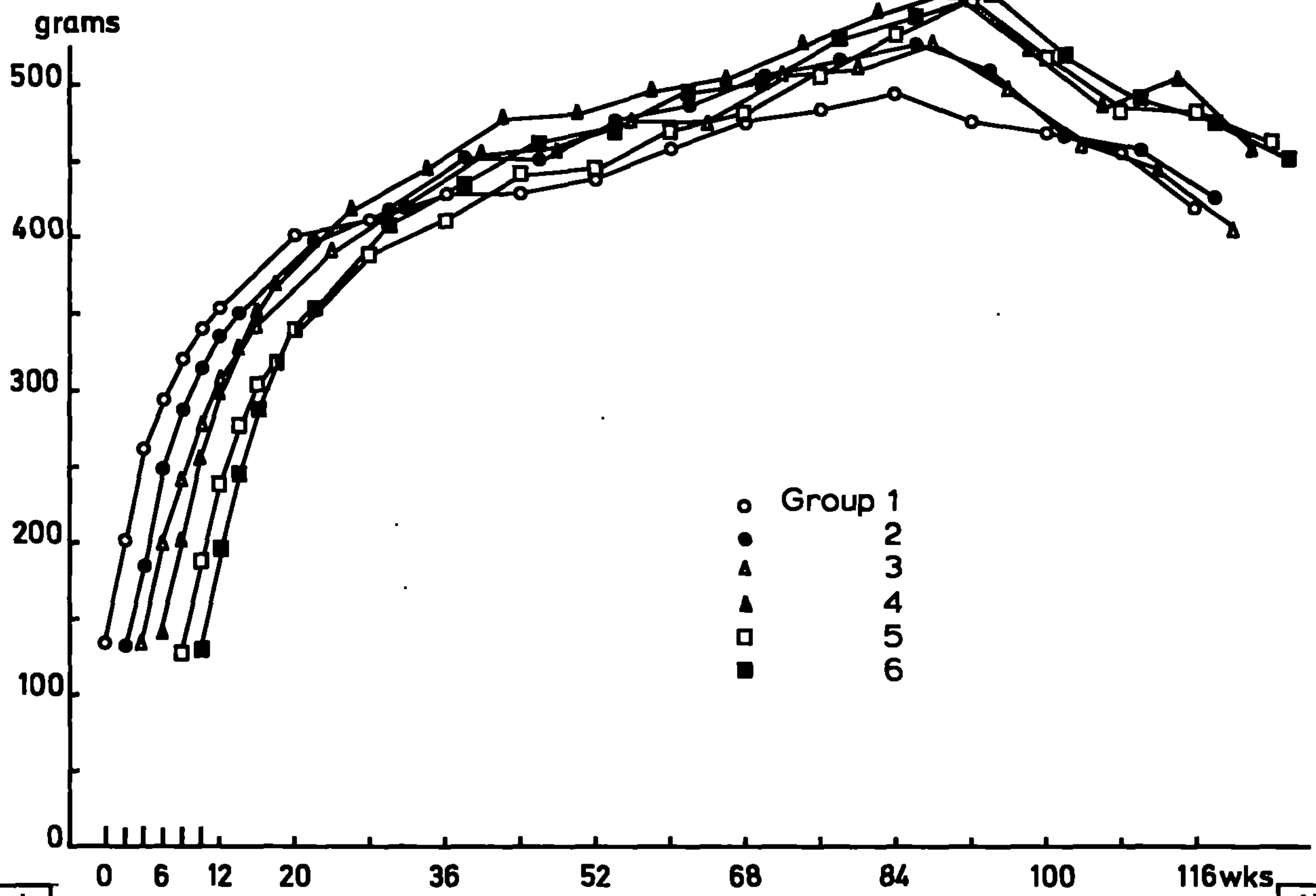
Fig. 1 growth of the female rats



R.I.V. Toxic.

17643

Fig. 2 growth of the male rats



R.I.V. Toxic. 17644

Fig. 3: Survival curves for female rats fed standard feed or feed supplemented with 35% autoclaved or irradiated pork

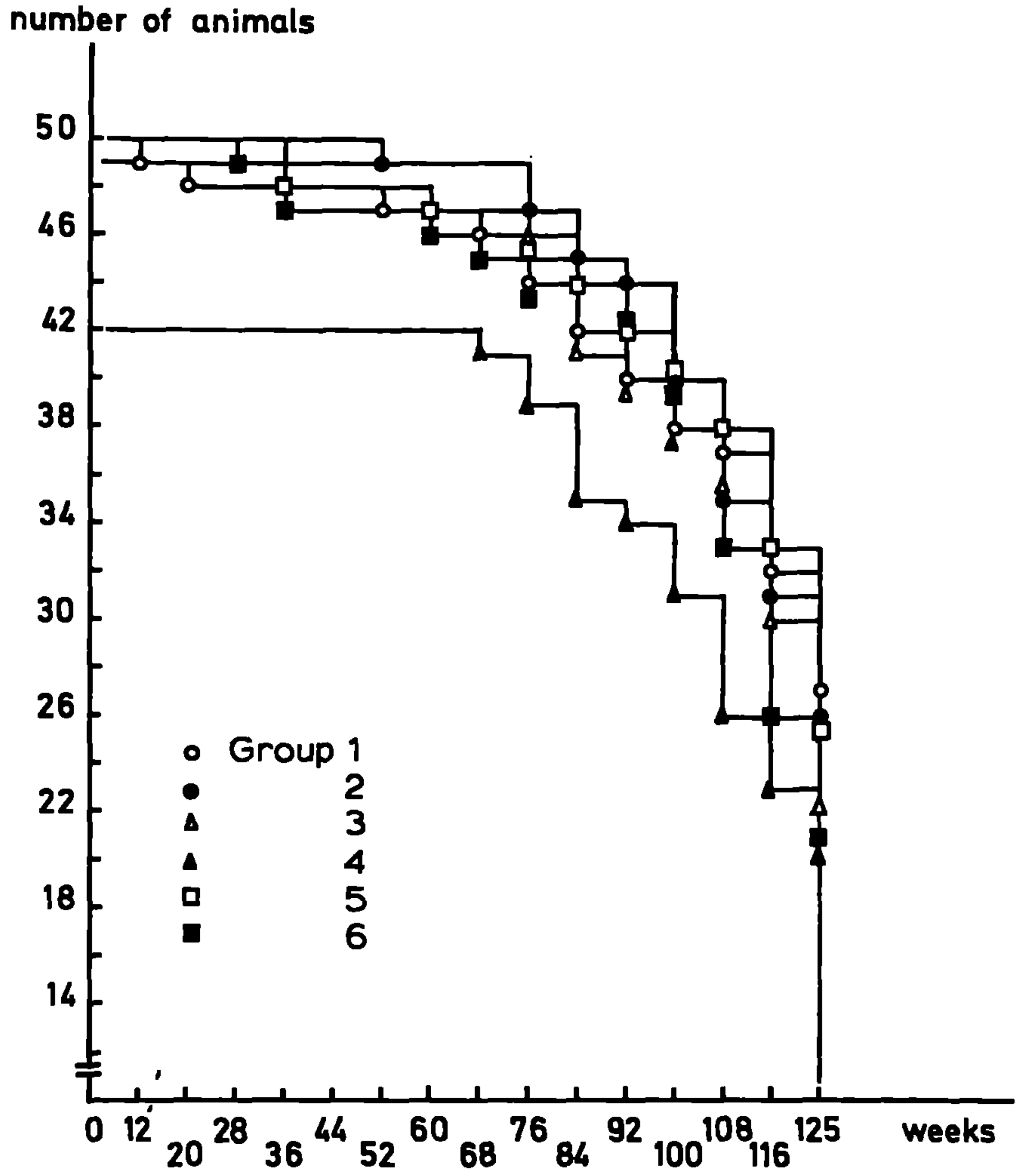
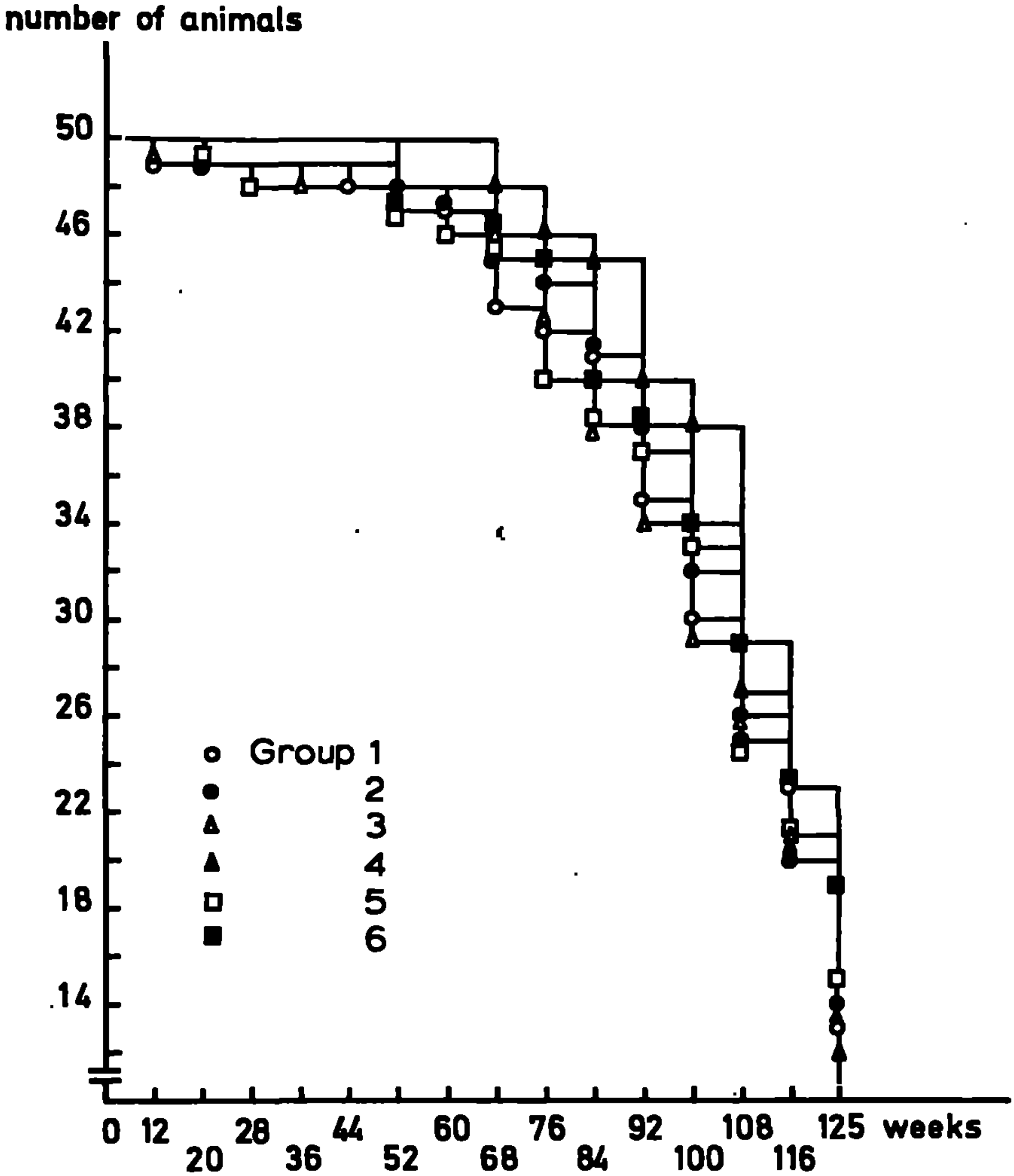


Fig. 4: Survival curves for male rats fed standard feed or feed supplemented with 35% autoclaved or irradiated pork meat



Chemical analysis

After irradiation or sterilization by autoclaving 2 to 4 tins of cured pork of every batch were analysed for moisture (dried at 105°C), fat (butyrometrically), protein (% nitrogen x 6.25), salt (% NaCl), nitrite (ppm $NaNO_2$) and phosphate (% P_2O_5).

In addition the percentage jelly set off was determined (table 2). These analyses were performed at the Central Institute for Nutrition and Food Research, Zeist.

Volatile N-nitrosamines in samples of pork were determined with GC-MS by the Laboratory for Chemical Food Analysis (6) (table 3).

Composition of the diets

The control rats, without pork in their diet, received the semi-purified standard diet Muracon SSP-Tox. The composition of this diet is given in table 3.

At the National Institute of Public Health (Bilthoven) the canned pork was mixed with animal diet in a weight ratio of 35%. Since the canned pork had a relatively high protein and salt content, a "supplemented diet" was used. The standard diet and the supplement diet (table 4) were specially compounded by Trouw and Co., Putten, The Netherlands.

At two time intervals (after 2 and 9 months) during the study the experimental diets were analysed by the "Rijks Landbouwproefstation", Maastricht, for the protein, fat (ether extract), crude fiber, moisture (80°C, vacuo) and chloride content. The results are given in table 5. Due to the high moisture content of the pork (about 75%) the moisture content of the experimental diets of the groups 2 until 6 is relatively high in comparison to the normal standard diet, whereas the protein, fat, cellulose and chloride concentration is lower.

The various diets were stored under refrigeration. The rats were given fresh feed daily.

Animals and diets

Male (weighed between 318 - 358 g) and female (194 - 214 g) SPF-derived Wistar rats Riv:TOX(M) were obtained from the Institute's own breeding colony. They were housed under conventional conditions in wire cages, two animals per cage. Food and drinking water were supplied ad libitum. The rats were divided randomly among six groups of 12 males and 24 females. The animals were fed an experimental diet according to the following scheme:

1. Standard : SSP (semi-synthetic purified)-Tox standard rat feed.
2. "Control autoclaved": 35% autoclaved meat, derived from pigs fed untreated conventional feed, with 200 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
3. Autoclaved : 35% autoclaved meat, derived from pigs fed autoclaved conventional feed, with 200 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
4. "Control irradiated": 35% 37 kGy irradiated meat, derived from pigs fed untreated conventional feed, with 50 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
5. Irradiated ~~(high)~~ low : 35% 37 kGy irradiated meat, derived from pigs fed 50 kGy irradiated conventional feed, with 50 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).
6. Irradiated ~~(low)~~ high : 35% 74 kGy irradiated meat, derived from pigs fed 50 kGy irradiated conventional feed, with 50 ppm nitrite, supplemented with 65% SSP-Tox-S (protein and salt adapted).

The six groups were given the 6 respective diets for two weeks. Thereafter each male was allowed to mate with two females for one week to produce a litter. With the offspring a chronic test was carried out. The parent generation was killed after weaning.

Experimental design

Upon weaning six groups, each comprising 50 male and 50 female rats (except group 3: 49 and group 4: 42 females) were used. They were chosen randomly from the dams.

In this experiment the rats were fed the same experimental diets for a period of 2½ years as the respective parent generation. The rats were housed in wire cages, two animals per cage. Food and drinking water were given ad libitum.

The animals were weighed weekly during the first 12 weeks and every 8 weeks thereafter. Food intake was recorded in week 1, 2, 5, 9, 12, 20, 36, 52, 68, 84, 101 and 116.

Blood samples were taken for haematological investigations from 10 female and 10 male animals from each group after 13, 26, 52, 104 weeks and at the end of the study. The haemoglobin concentration, haematocrit value, concentration of erythrocytes and concentration and differential counts of leucocytes were determined. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated*. Serum urea concentration and activity of glutamic-pyruvic-transaminase (SGPT), glutamic-oxalacetic transaminase (SGOT) and alkaline phosphatase activity (Alk.Pase) were determined in 5 female and 5 male rats of each group after 26 and 104 weeks. The glucose concentration in the serum was estimated after 104 weeks. Semi-quantitative urinalysis of pH, protein, glucose, keton bodies, bilirubin and blood with Bililabstix was carried out after 26 and 104 weeks. After 104 weeks the concentration of creatinine and protein in urine was determined (7).

All animals were inspected regularly. Animals becoming moribund were autopsied. After 125 weeks the surviving animals were killed with carbon dioxide. Heart, brain, liver, kidneys, spleen, adrenals, thyroid, pituitary, coecum, uterus, ovaries, testes and prostate were dissected and weighed. All animals that died or were killed were autopsied and all organs were fixed in buffered 4% formalin (except brain in 10%) or Bouin solution for histopathological examination. The organs weighed as well as lungs, pancreas, mesenteric lymph nodes, salivary glands, stomach, duodenum, ileum, jejunum, colon, rectum, urinary bladder, spinal cord, n.ischiadicus and gross lesions were studied histopathologically.

Statistical analysis

Significancy of differences in group means - or geometric means - were established by the Student's t test (two-sided). In case of insufficient homogeneity of variances, the Welsh correction with respect to the degrees of freedom was applied.

* $0{,}01 < P < 0{,}05$ in comparison to group 2

** $0{,}001 < P < 0{,}01$ in comparison to group 2

*** $P < 0{,}001$ in comparison to group 2

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