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A SUBCHRONIC TOXICITY STUDY OF ROKANOL B-2 IN RATS

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Abstract: The toxicity of Rokanol B-2 was assessed following its administration to rats via oral gavage. A preliminary study of acute toxicity was performed to determine suitable dosing regimen/dose levels for future repeated-dose toxicity studies. For this purpose rats (15 males and 7 females) received single doses of 2000, 1500, 1000 or 500 mg/kg and were killed after 2 or 14 days. No deaths occurred during the observation time. Dose-related irritation to the stomach mucosa was found. For the subchronic toxicity assessment, Rokanol B-2 was administered at daily doses of 8, 40 or 200 mg/kg to 59 male and 56 female Wistar rats by oral gavage, 5 days per week for 90 days. An interim experiment was performed after 28 days of dosing. Five animals/sex/group were terminated and necropsied. Blood samples for clinical chemistry and haematology were obtained and lungs, heart, adrenals, kidneys, liver, spleen, stomach, intestine, testes (males), uterus and ovaries (females) were weighed and prepared for histopathological examination. After 90 days of dosing all remaining animals were killed and necropsied. Blood samples were taken for evaluation of haematology and clinical chemistry, and selected organs (same as above) prepared for subsequent histological examination. In the high-dose group (200 mg/kg/day) a statistically significant reduction in body weight gain and food consumption, an increased weight of the liver in the males and disturbances in haematological parameters in the females were observed.

Ulcerations of the mucous membrane of the stomach and hyperkeratosis, and in a few cases, pseudopapillomatous epithelial proliferation of foregaster and exudate in the submucosa of the stomach were noted. In the mid-dose group (40 mg/kg/day) some disturbances in hematological parameters in females and histopathological changes in rats of both sexes similar, but to a lesser degree, to changes observed in high-dose animals were indicated. In the low-dose group (8 mg/kg/day) no significant treatment-related effects were observed. The results of this study indicate that Rokanol B-2 possessed an overall low degree of systemic toxicity when administered orally to rats for 90 days. The NOAEL, observed in this study, was estimated as 8 mg/kg/day.

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INTRODUCTION

Rokanol B-2 is a non-ionic detergent used mainly for preparation of household detergents. It is manufactured by the addition of propylene oxide and ethylene oxide to a fatty alcohol and has the chemical formula

$$RO\ (CH2\ CH2\ O)8\ (\underset{\underset{CH3}{|}}{CH2}\ CH2\ O)6H$$

where R is a fatty alcohol group.

Rokanol B-2 is a straw-coloured liquid of dense oleic consistency with the density of 0.98 g/cm^3. It is insolube in water but soluble in benzene, ethanol, olive oil and in polypropylene glycol.

In industrial conditions there is little or no risk of poisoning by inhalation due to a low vapour pressure of Rokanol B-2. This type of non ionic detergents are characterized by low oral acute toxicity but strong irritation activity for the skin (4,7,13). The unpublished data provided by the manufacturer, showed that it is a compound of low acute toxicity; oral LD_{50} in rats was found to be above 2000 mg/kg. Skin and eye irritation studies in rabbits indicated that it was slightly irritant. Rokanol B-2 did not exhibit genotoxic activity in Ames test, micronucleus test *in vivo* and sister chromatid exchange test (9).

This compound is used in high volume in the preparation of different types of household detergents widely used in Poland. The present study was undertaken in view of the limited data on toxicity of non-ionic detergents, and regarding the toxicity of Rokanol B-2. The specific purpose was to evaluate its subchronic toxicity when administered orally to rats during a 90-day gavage study.

MATERIALS AND METHODS

Test material

Rokanol B-2 was obtained from the Organika Rokita, Brzeg Dolny, Poland, and according to the supplier its purity was 99%. No impurities were identified.

Animals and treatment groups

Wistar rats from the Nofer Institute of Occupational Medicine breeding colony were used. Animals were quarantined for 2 weeks prior to the initiation of the study. Test animals were approximately 6 weeks old at the start of dosing. The mean initial body weight was 174 $\pm$ 12 and 145 $\pm$ 8 for male and female rats, respectively. Any animals determined to be unsuitable for the study because of poor health, unsuitable body weight etc., were excluded. The remaining animals were selected for the study using a computer-generated body weight sorting program. The rats were housed in polypropylene cages with sawdust as bedding, five animals/sex in each. Room temperature and humidity were maintained at 22 $\pm$ 2°C and 30–70% on a 12 hr-light-dark cycle. Food (Murigran pelleted rodent chow, Fodder Plant Motycz, Poland) and water were provided *ad libitum*.

The preliminary acute toxicity study was performed on 22 rats (15 males and 7 females) in order to determine an appropriate study design and dosing regimen for

further repeated-dose toxicity studies. The treated rats received single doses of either 2000, 1500, 1000 or 500 mg/kg b.w. Control rats, 2 males and 2 females received olive oil at a dose of 2.5 ml/kg b.w. Animals were killed and necropsied after 2 (9 males) or 14 days (4 males and 5 females) of observation; the controls (2 males and 2 females) were killed and necropsied after 14 days of observation.

The main experiment comprised four groups of 10 male and 10 female rats with an additional 2 males and 2 females in the high dosage group (less then 1/10 LD_{50}). The animals were dosed once daily, 5 days per week for 13 weeks (90 days). Five animals/sex/group were killed after 28 days (interim termination) and the remaining animals were killed after 90 days. The dose was formulated as a solution in olive oil and was administered in a limited volume of 2.5 ml/kg by oral gavage with a stainless-steel ball-tipped feeding canula attached to 5-ml disposable syringe. Treated rats received Rokanol B-2 at dosages of 8 mg/kg (low-dose), 40 mg/kg (mid-dose) and 200 mg/kg (high-dose). The control rats received olive oil at the same dose volume.

Observations and biological measurments

Observations were made daily for overt signs of toxicity. Body weights of animals were recorded prior to the first dosing and weekly thereafter during the test period. Food consumption was measured weekly during the course of the study.

Acute toxicity

In the preliminary study of acute toxicity, histopathological examinations were performed on the following organs: lungs, heart, liver, kidneys, spleen, stomach, small and large intestine, testes (in male rats), uterus and ovary (in female rats). The tissues and organs listed above were preserved in 10% buffered formalin, routinely processed, stained with haematoxylin and eosin and evaluated microscopically. The presence of neutral lipids was assessed in the liver and kidneys, by incubation of frozen sections of these organs in saturated Fat Red 7B solution in 70% isopropanol (2,14).

Interim termination

Five animals per sex per group were killed and necropsied after 28 days of dosing. Organ weights were obtained for liver, spleen, kidneys, adrenals and testes/ovaries. All necropsied animals were evaluated for alterations in haematological and clinical chemistry parameters, gross lesions, organ weights and histopathological studies. Blood samples were collected for the serum determination of alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, gamma-glutamyl transpeptidase, alkaline phosphatase, blood urea nitrogen, total protein, albumin, globulin, glucose, total bilirubin, triglycerides, cholesterol, sodium, potasium, chloride, calcium and inorganic phosphorus. Haematologal analysis included erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (Ht), total white blood cells (WBC) and differential leucocyte counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocyte count and platelet count. The following organs and tissues were evaluated histopathologically: lungs, heart, liver, kidneys, adrenal gland, spleen, stomach, duodeum, pancreas, thyroid, small and large intestine, testes and epididymides or uterus and ovaries.

A 90-day study

On day 91 of the study all remaining animals were killed and a complete necropsy was performed. Blood samples were obtained from all groups for routine haematology and clinical chemistry determinations as described above. Organ weights were obtained for liver, spleen, kidneys, adrenals and testes/ovaries. The organs and tissues in low and mid dose animals, evaluated histopathologically, were the same as in the interim termination. In addition, the following tissues were examined from the control and high dose groups of the animals: brain, cerebellum, thyroids and parathyroids, salivary gland, thymus, oesophagus, mediastinal lymph nodes, urinary bladder, prostate gland, seminal vesicles, mammary glands, eye with optic nerve, lacrimal glands, skin, femoral muscles, femur with joint, spinal cord and spinal medulla. They were preserved and prepared for histopathological examinations as described above.

Statistical analyses

With the exception of body weight data, all parameters from the treated groups were compared statistically to those of the control group using the following tests: Bartlett's test of homogeneity of variance was used to determine if the groups had equivalent variances at the $p < 0.01$ level (17). If the variance was not significantly different, the groups were compared using a standard one-way analysis of variance (ANOVA) (16). If significant differences between the means were indicated, Dunnett's test was used to determine which treatment groups differed from the controls (17,18). If the groups showed no equivalent variances at the $p < 0.01$ level, then the Kruskal-Wallis test was used to assess differences in group means. If the means were different, Dunn's summed rank test was used to determine which treatment group differred significantly from the control (8). A dose-dependent trend was assessed statistically by linear regression analysis (1). Body weight data for each sex was analyzed by one-way classification analysis of covariance (ANCOVA), using preexposure (day 0) weights as the covariate. All these procedures were carried out by means of the TOXSTAT program (10). Frequency of pathological lesions in the stomach was evaluated by the Fisher exact test (16).

RESULTS

Preliminary study

There were no deaths during the observation period. In-life clinical observations were unremarkable. Gross necropsy observations showed marked congestion of the stomach and the intestine in rats given the compound in doses exceeding 1000 mg/kg. In rats killed 2 days after dosing with 1500-2000 mg/kg of Rokanol B-2, the following histological changes were noted: erosive gastritis of the foregaster and glandular mucosa of stomach, reactive proliferation and hyperkeratosis of the foregaster epithelium, and submucosal oedema. In a few animals, proliferation of goblet cells in the stomach, duodenum and small and large intestine, and diffuse steatosis of the liver parenchyma, were observed. Lower dosages of Rokanol B-2 (i.e. 1000 mg/kg and 500 mg/kg) induced hyperkeratosis in the forestomach mucosa and a multifocal increase in the thickness of the epithelium, especially of the basal layer.

Table 1. Effect of Rokanol B-2 on haematological parameters (gavage, 28 days)

Parameters[a]	Treatment group				Linear regression analysis
	Control n = 5	8 mg/kg n = 5	40 mg/kg n = 5	200 mg/kg n = 5 females n = 3 males	
Haematocrit (%)					
Males	53.7±2.3	52.7±1.5	51.3±11.3	56.3±2.7*	**P = 0.0131**
Females	49.3±2.2	47.7±2.1	46.6±1.4*	49.1±3.7	**P = 0.0239**
Haemoglobin (g/dl)					
Males	16.1±1.7	15.8±2.0	14.8±1.5	14.8±2.8	**P = 0.0299**
Females	14.4±1.1	13.0±2.1	10.4±0.7**	10.4±1.0**	**P = 0.0001**
RBC (× 106/mm^3)					
Males	10.53±1.38	10.86±1.08	10.90±1.10	10.57±0.94	**P = 0.2516**
Females	9.59±1.30	8.46±1.35	7.82±1.56*	7.66±0.83**	**P = 0.0076**
MCV (m^3)					
Males	51.8±7.6	48.9±4.8	47.5±4.9	53.7±5.6	**P = 0.0579**
Females	52.3±7.8	58.0±11.9	61.9±13.2	64.8±9.4*	**P = 0.0042**
MCH (pg)					
Males	15.5±2.5	14.8±2.7	13.7±2.0	14.1±3.0	P = 0.2747
Females	15.2±2.3	15.6±2.5	13.8±2.9	13.7±2.2	P = 0.9993
MCHC (%)					
Males	30.2±4.2	30.0±3.6	28.9±3.1	26.4±4.9	**P = 0.0000**
Females	29.2±2.0	27.4±5.1	22.3±1.5**	21.3±2.4**	**P = 0.0000**
Reticulocyte (%)					
Males	8.0±7.6	8.5±3.8	7.8±3.5	8.3±3.9	P = 0.6680
Females	15.6±5.0	8.2±3.8*	8.6±2.0*	4.6±2.3**	**P = 0.0000**
Leucocyte (× 10^3/mm^3)					
Males	13.91±2.67	12.74±4.40	15.18±2.75	17.18±2.75	**P = 0.0073**
Females	11.22±2.81	10.02±1.81	8.06±1.57*	10.87±3.73	P = 0.3312
Neutrophils (%)					
Males	15.3±5.7	18.3±7.8	14.6±4.0	20.2±7.7	P = 0.0705
Females	11.6±5.4	11.9±5.0	15.7±4.3	28.1±14.1**	**P = 0.0000**
Lymphocytes (%)					
Males	82.9±7.5	79.1±9.0	80.2±5.0	74.4±9.5	P = 0.0620
Females	87.0±5.8	83.2±8.1	79.5±4.9	68.0±15.1**	**P = 0.0000**
Platelets (× 10^3/mm^3)					
Males	308.6±40.5	316.9±58.5	352.3±70.3	254.6±51.8	**P = 0.0020**
Females	283.3±38.7	276.4±55.6	249.5±51.2	221.9±54.9*	**P = 0.0067**

[a] other parameters not included in the table were not significantly different from controls,

P probability for a concentration-dependance assessed by Jonckheere test,

*,** statistical significance between the exposure group and the control at $p < 0.05$ and $p < 0.001$, respectively.

Results expressed as a mean ±SD.

In the intestines no significant changes were noted. Periportal steatosis was observed in the liver of rats treated with 1000 mg/kg, and vacuolisation of hepatocytes was noted in rats treated with 500 mg/kg of Rokanol B-2. No significant changes were noted in the organs of rats sacrificed 14 days after treatment with dosages of 500, 1000, 1500, 2000 mg/kg; however, an increased thickness of the foregaster epithelium was observed in one male treated with 2000 mg/kg of Rokanol B-2.

Interim experiment

Mortality. Two male rats receiving 200 mg/kg Rokanol B2 died on days 6 and 9 of the study.

In-life observations. There were no clinical signs worthy of note.

Body weights and food consumption. Mean food consumption values were significantly lower in males receiving 200 mg/kg (data not shown). In all treated groups body weight gains after 28 days did not differ significantly from the controls.

Organ weights. All organ weights in the treated groups were comparable to control values.

Clinical chemistry and haematology. Statistically significant increase in urea, glucose and cholesterol levels were observed only in males receiving 200 mg/kg (data not shown). Mean haematology values for the treated and control groups are shown in Table 1. Changes observed included dose-dependent decrease in haemoglobin concentration, MCHC and platelet counts in both sexes and decrease in erythrocytes counts, reticulocyte counts and percentage of lymphocyte, increase in hematocrit in males, and increase in MCV and percentage of neutrophils in females.

Necropsy and histopathology. Hyperkeratosis and ulceration as well as an exudate in the submucosal area of the stomach were observed in animals receiving 200 or 40 mg/kg (Table 2). Microscopic evaluation demonstrated pseudopapillomatous epithelium proliferation of the foregaster in some of the rats treated with 200 mg/kg.

Table 2. The frequency and type of changes in foregaster of rats exposed to Rokanol B-2 (by gavage, 28 days)

Dose mg/kg	No of rats	Epithelium proliferation	Papillomatus proliferation	Hyper keratosis	Submucous oedema	Ulceration	No of rats with changes	No of rats without changes
200	3 M	3	2	2	0	0	3	0
200	5 F	4*	2	3	1	2	4*	1
40	5 M	2	0	2	2	0	3	2
40	5 F	1	0	1	2	0	2	3
8	5 M	0	0	0	0	0	0	5
8	5 F	0	0	0	0	0	0	5
0	5 M	0	0	0	0	0	0	5
0	5 F	0	0	0	0	0	0	5

M – males; F – females. 0 – no changes observed. * – significantly different from the control $p < 0.05$.

After 90 days

Mortality. In the high-dose group mortality of rats was 25% for males and 33% for females.

In-life observations. After 50 days of dosing rats receiving 200 mg/kg exhibited occasional salivation and piloerection. In addition, 5 rats (3 male, 2 female) receiving 200 mg/kg showed abdominal swelling a few days prior to death. No significant clinical signs were observed in animals treated with 40 or 8 mg/kg.

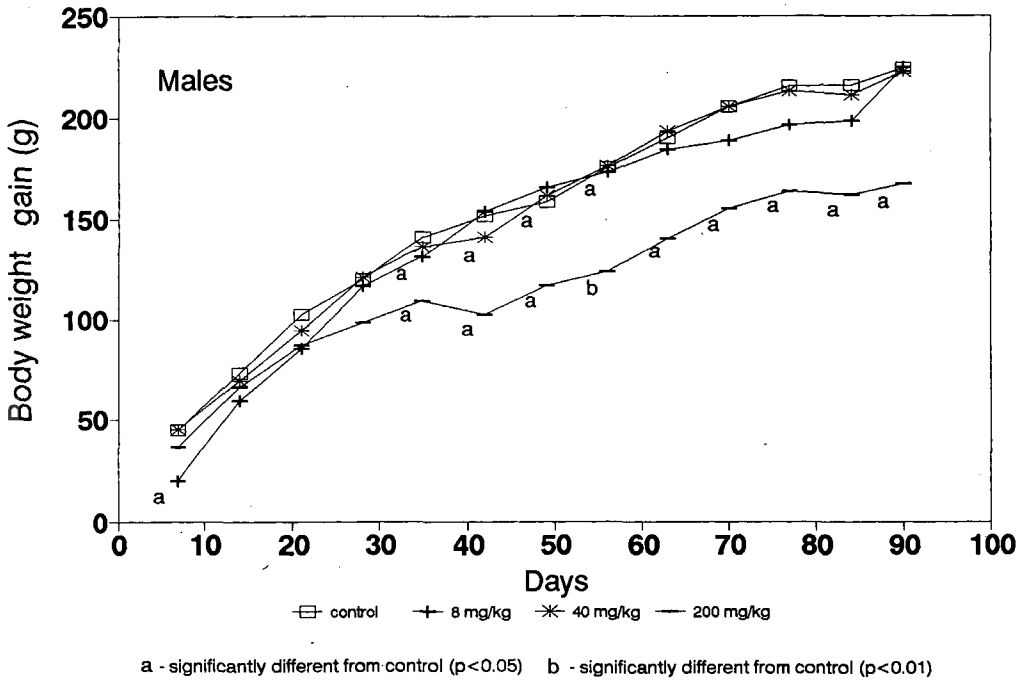

Fig. 1. Changes of body weight gain (g) of male rats dosed with Rokanol B-2.

Body weight and food consumption. In general, mean food consumption was significantly lower in the high-dose males only (data not shown). Group mean body weight gain for males receiving 200 mg/kg was significantly lower in comparison with the controls from Day 35 of dosing until termination (Fig. 1, Table 3). Body weight gains of female rats in all treated groups did not differ significantly from the controls (Fig. 2).

Organ weights. Mean organ weights for male and female rats are presented in Table 3. Absolute liver weight in females receiving 200 mg/kg was elevated when compared to that of the controls, although the difference was not statistically significant. Relative liver weight (organ weight to body weight ratio) in males receiving 200 mg/kg was significantly elevated when compared to that of the controls. Additionally, a dose related increase in relative liver weight was observed as assessed with linear regression analysis ($p < 0.001$). There were no significant intergroup differences in absolute testes weight, however, relative values showed

Table 3. Effect of Rokanol B-2 on organ and terminal body weight after 90 days

Parameters	Treatment group				Linear regression analysis
	Control	8 mg/kg	40 mg/kg	200 mg/kg	
Males	n = 10	n = 10	n = 10	n = 9	
Terminal body weight (g)	399 ± 42	405 ± 23	396 ± 50	339 ± 36*	**P = 0.0014**
Absolute organ weight (g)					
Liver	9.59 ± 1.20	9.82 ± 0.87	9.19 ± 1.29	9.45 ± 0.88	P = 0.4900
Spleen	0.736 ± 0.136	0.717 ± 0.155	0.667 ± 0.113	0.640 ± 0.182	P = 0.5354
Kidneys	2.49 ± 0.49	2.33 ± 0.23	2.37 ± 0.56	2.05 ± 0.46	P = 0.1198
Adrenals	0.053 ± 0.009	0.052 ± 0.006	0.052 ± 0.011	0.049 ± 0.015	P = 0.3397
Testes	3.76 ± 0.31	3.67 ± 0.27	3.78 ± 0.21	3.45 ± 0.35	P = 0.6810
Relative organ weight (g/100 g b.w.)					
Liver	2.41 ± 0.16	2.42 ± 0.15	2.32 ± 0.10	2.82 ± 0.22**	**P = 0.0000**
Spleen	0.184 ± 0.026	0.176 ± 0.032	0.169 ± 0.022	0.189 ± 0.037	**P = 0.0471**
Kidneys	0.629 ± 0.130	0.576 ± 0.047	0.598 ± 0.121	0.606 ± 0.053	P = 0.3138
Adrenals	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.015 ± 0.004	P = 0.3598
Testes	0.949 ± 0.093	0.908 ± 0.079	0.967 ± 0.101	1.053 ± 0.209	**P = 0.0002**
Females	n = 10	n = 10	n = 10	n = 8	
Terminal body weight (g)	227 ± 16	216 ± 19	236 ± 24	232 ± 33	P = 0.9567
Absolute organ weight (g)					
Liver	6.00 ± 0.68	5.98 ± 0.48	6.00 ± 0.75	6.32 ± 0.90	P = 0.1591
Spleen	0.518 ± 0.089	0.458 ± 0.061	0.537 ± 0.065	0.570 ± 0.089	P = 0.1380
Kidneys	1.32 ± 0.15	1.34 ± 0.17	1.39 ± 0.17	1.38 ± 0.17	P = 0.8878
Adrenals	0.062 ± 0.011	0.069 ± 0.011	0.062 ± 0.007	0.062 ± 0.008	P = 0.4259
Ovarium	0.083 ± 0.016	0.086 ± 0.013	0.089 ± 0.025	0.084 ± 0.027	P = 0.9409
Relative organ weight (g/100 g b.w.)					
Liver	2.65 ± 0.23	2.77 ± 0.20	2.55 ± 0.19	2.75 ± 0.43	P = 0.0717
Spleen	0.228 ± 0.030	0.212 ± 0.030	0.228 ± 0.020	0.250 ± 0.050	P = 0.0912
Kidneys	0.584 ± 0.042	0.619 ± 0.063	0.592 ± 0.046	0.598 ± 0.037	P = 0.7401
Adrenals	0.027 ± 0.004	0.032 ± 0.006	0.027 ± 0.003	0.027 ± 0.004	P = 0.4765
Ovarium	0.036 ± 0.006	0.040 ± 0.007	0.038 ± 0.009	0.036 ± 0.009	P = 0.9616

*,** statistical significance between the exposure group and the control at $p < 0.05$ and $p < 0.001$, respectively.
P probability for a concentration-dependance assessed by Jonckheere test.
Data represents a mean ± SD.

a dose related increase ($p < 0.001$). All other organ weights in the treated groups were comparable to control values.

Clinical chemistry and haematology. Decrease in a haematocrit value, MCV, MCH and lymphocytes percentage, and an increase in RBC in animals treated with 200 or 40 mg/kg, as well as neutrophil percentage in the high-dosed females were noted. Haematology results are presented in Table 4. For the majority

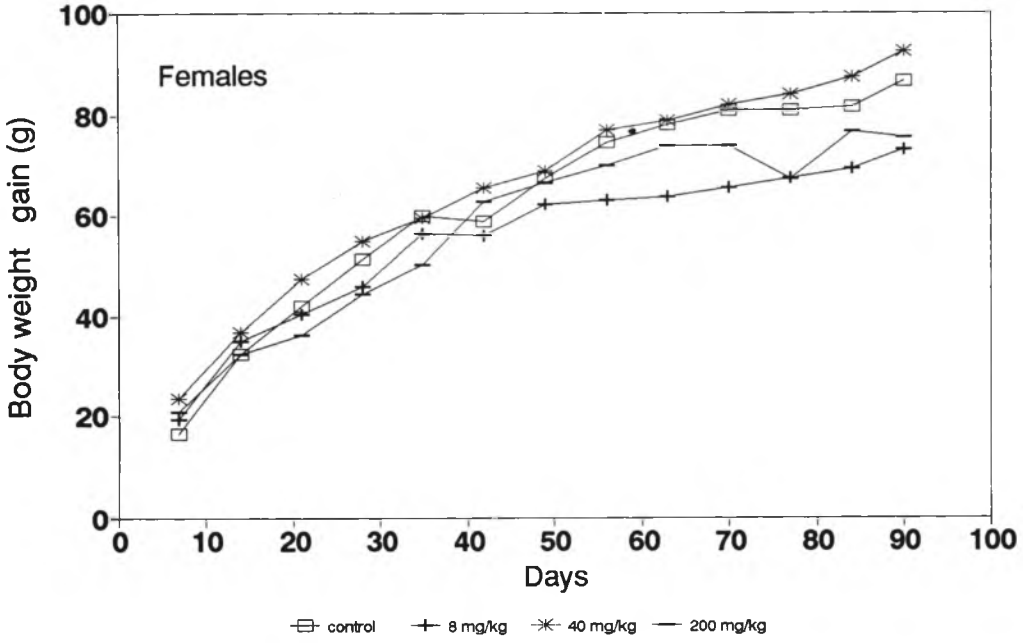

Fig. 2. Changes of body weight gain (g) of female rats dosed with Rokanol B-2.

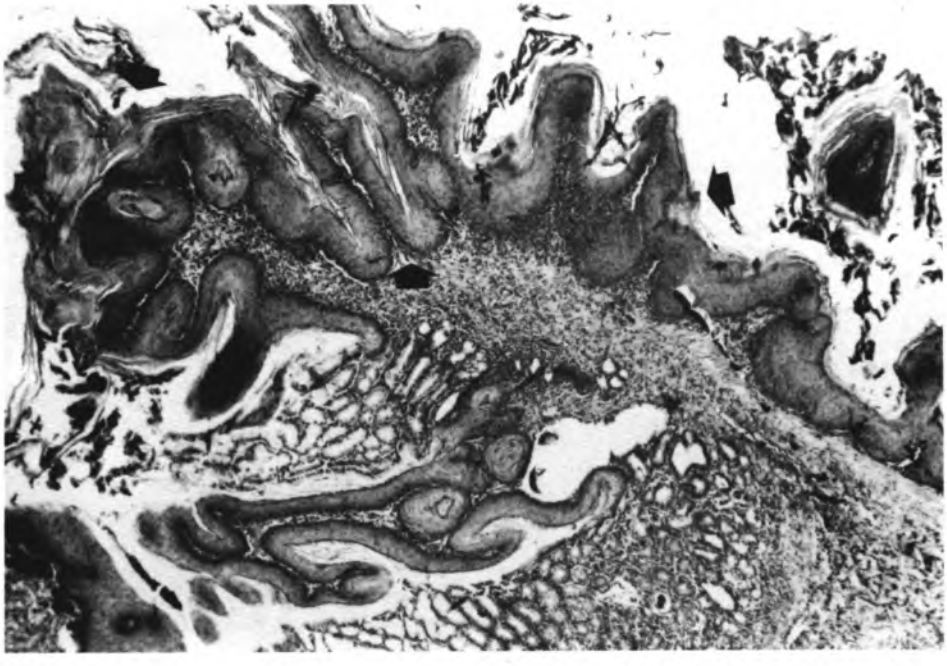

Fig. 3. Foregaster of the female rat killed after 28 days of gavage of Rokanol B-2 in a dose of 200 mg/kg/day. Hyperkeratosis and papillomatous proliferaction of the foregaster mucosa (arrows). H & E staining. (Magn. 30 x).

Table 4. Effect of Rokanol B-2 on haematological parameters (gavage, 90 days)

Parameters[a]	Treatment group				Linear regression analysis
	Control n = 10	8 mg/kg n = 10	40 mg/kg n = 10	200 mg/kg n = 9 males n = 8 females	
Haematocrit (%)					
Males	53.5±1.3	52.1±2.2	55.1±3.2	55.6±3.2	P = 0.0780
Females	49.8±1.2	48.6±1.9	47.3±2.0	46.5±2.6*	**P = 0.0029**
Haemoglobin (g/dl)					
Males	17.2±0.9	17.4±0.6	16.7±1.2	16.5±1.4	P = 0.6885
Females	16.1±1.2	16.0±1.0	17.6±2.5	16.2±1.4	P = 0.0792
RBC ($\times 10^6$/mm^3)					
Males	10.41±1.53	9.91±1.70	9.22±1.23	10.01±1.11	P = 0.8061
Females	7.30±0.63	7.53±0.46	8.35±0.84*	8.72±1.07**	**P = 0.0168**
MCV (m^3)					
Males	52.4±8.1	54.1±10.3	60.6±7.8	56.2±8.0	P = 0.8774
Females	68.7±6.9	64.8±4.9	57.1±6.0**	54.0±7.0**	**P = 0.0008**
MCH (pg)					
Males	16.8±2.4	18.1±3.1	18.4±2.6	16.8±3.0	P = 0.8082
Females	22.2±2.9	21.3±1.9	21.2±3.4	18.8±2.1	**P = 0.0036**
MCHC (%)					
Males	32.2±2.2	33.5±1.7	30.5±3.7	29.8±3.1	P = 0.4299
Females	32.3±2.4	32.8±1.6	37.1±4.6*	34.9±2.5	P = 0.6453
Reticulocyte (%)					
Males	3.5±1.4	4.1±3.8	4.6±1.9	4.8±4.1	P = 0.7832
Females	6.4±3.2	5.9±2.2	6.5±4.5	4.5±3.3	P = 0.1301
Leucocyte ($\times 10^3$/mm^3)					
Males	10.96±2.13	10.37±3.97	11.54±3.73	11.50±2.81	P = 0.0547
Females	11.10±3.37	8.70±1.51	9.89±2.41	8.61±2.06	P = 0.0795
Neutrophils (%)					
Males	17.6±5.9	21.2±11.8	25.9±9.7	25.4±17.7	P = 0.5864
Females	15.7±9.7	18.6±7.2	15.0±4.5	28.3±10.0	**P = 0.0002**
Lymphocytes (%)					
Males	79.7±6.5	76.7±12.1	69.8±11.7	71.6±19.2	P = 0.7083
Females	81.8±12.4	77.5±7.4	82.1±5.6	67.6±11.5*	**P = 0.0013**
Platelets ($\times 10^3$/mm^3)					
Males	320.7±49.9	360.0±131.2	237.6±77.3*	233.2±47.2*	**P = 0.0444**
Females	268.5±45.2	217.7±39.7	262.8±38.2	224.4±30.7	**P = 0.0471**

[a] Other parameters not included in the table were not significantly different from controls;

*,** statistical significance between the exposure group and the control at $p < 0.05$ and $p < 0.001$, respectively.

P probability for a concentration-dependance assessed by Jonckheere test.

Data represents a mean ±SD.

of serum chemistry values measured, there were no significant differences in treated animals as compared to the controls. Single instances of statistically significant deviations from the controls were observed for total cholesterol concentration and inorganic phosphorus in females receiving 200 mg/kg. As the observed differences were small and not evident in other treatment groups, they were judged to be of no toxicological relevance, although in some cases they were dose-dependant.

Necropsy and histopathology. Gross necropsy observations at the time of termination showed congestion of the organs and pneumatosis of the small intestine in animals receiving 200 mg/kg. Microscopic evaluation showed pseudo-papillomatous epithelium proliferation of the foregaster in some of the rats treated with 200 mg/kg, reactive squamous epithelial proliferation (hyperplasia) and organization exudate in the submucosal region in males and females treated with 200 or 40 mg/kg (Figs 3 and 4). The increased incidence of the observed changes in the

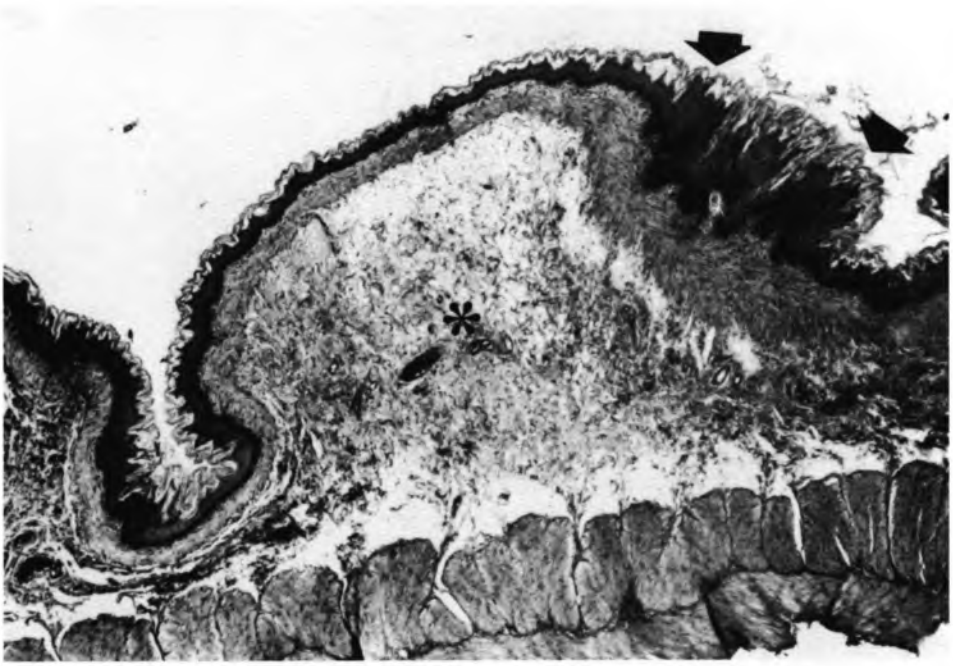

Fig. 4. Micrograph of the stomach of female rat killed after 90-days of gavage of Rokanol B-2 in a dose of 200 mg/kg/day. Hyperkeratosis and papillomatous proliferaction of the foregaster mucosa (arrows). The subacute ulcer of mucosa is also seen (asterisk). H & E staining. (Magn. 30 x).

stomach in the Rokanol B2 treated groups was statistically significant in comparison with the control group (Fisher exact test, $p < 0{,}05$ and $p < 0{,}005$) (Table 5). These differences were not observed in animals receiving 8 mg/kg or control animals. An increased number of goblet cells was observed in the epithelium of the small intestine of animals receiving 200 mg/kg. An increased variation in the size of hepatocyte nuclei was noted in animals receiving 200 mg/kg and scattered mononuclear interstitial infiltrations were also observed in the liver. In other tissues examined histopathological changes were limited to naturally occurring lesions.

Table 5. The frequency and type of changes in foregaster of rats exposed to Rokanol B-2 (gavage, 90 days)

Dose mg/kg	No of rats	Epithelium proli-feration	Papillo-matus proli-feration	Hyper keratosis	Sub-mucous oedema	Ulce-ration	No of rats with changes	No of rats without changes
200	9 M	9***	3	9**	7**	0	9**	0
200	8 F	8**	3	8**	4	0	8**	0
40	10 M	8**	0	8**	1	0	8**	2
40	10 F	8**	0	8**	1	0	8**	2
8	10 M	0	0	0	0	0	0	10
8	10 F	0	0	0	0	0	0	10
0	10 M	0	0	0	0	0	0	10
0	10 F	0	0	0	0	0	0	10

M – males; F – females.
0 – no changes observed.
* – significantly different from the control $p < 0.05$.
** – significantly different from the control $p < 0.001$.

DISCUSSION

These studies were performed in oder to assess the systemic toxicity of Rokanol B-2 when administerd subchronically via oral gavage.

In general, the results indicated a low degree of systemic toxicity for Rokanol B-2, particularly considering the comparatively large doses administered. Several treatment-related effects were observed, predominantly in the high-dose group (200 mg/kg). These effects consisted of slight reductions in food consumption and body weight gain in males, of increased relative liver weight, disturbances in some haematological parameters, and histopathological changes in the stomach.

The reduction in food consumption and body weight gain in rats were related to the administered dose and may have been caused by an irritant effect of Rokanol B-2 on the mucous membrane of the alimentary tract (3).

In the absence of any histological correlate and any alterations in serum chemistry parameters, the increase in liver weight observed in animals treated with 200 mg/kg is considered to be a compensatory response due to an increased metabolic work load (12). The increased variation in the size of hepatocyte nuclei may also be related to this metabolic adaptation (12).

The changes in haematological parameters were non-progressive and were probably associated with haemorrhage from the stomach mucosal ulceration observed histopathologicaly, however, there were no significant differences in the reticulocyte count, and an adaptive response of the bone marrow cannot be excluded.

The increased number of neutrophils observed in females and of leucocytes in males is most probably related to the reactive inflammatory process in the intestine as a result of a local irritant reaction of the Rokanol B-2 (3).

It might be concluded that Rokanol B-2 has marked irritant properties when administered orally. These properties are responsible for the destructive (ulcerations) and proliferative changes observed in the foregaster mucosa during 1 and 3 months of the treatment with high doses. Stomach ulcerations and stress related to it might be a possible cause of death observed in high-dosed animals.

The observed changes in the submucosa of the stomach in the form of exudate organization are probably irreversible. An increased mucine substance production by goblet cells also suggests local reactive responses to Rokanol B-2 as a toxic irritant agent (3,5,6). It is also known that prolonged exposure to irritative compounds may lead to proliferative changes (3,15).

Our subchronic oral studies performed on rats show that administration of Rokanol B-2 at doses of 8 mg/kg/day resulted in NOAEL (no-observed-adverse-effect-level). Administration of 40 mg/kg/day was LOEL (the lowest-observed-effect-level) for histopathological changes (local stomach mucosa irritation) and related disturbances in haematological parameters.

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