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# Acute and Subacute Oral Toxicity of AHR-2438B, a Purified Sodium Lignosulphonate, in Rats

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**Abstract**—The acute oral $LD_{50}$ of AHR-2438B in rats was found to be >40 g/kg. In subacute toxicity tests, rats were given AHR-2438B in the drinking-water at levels of 0 (control), 0·025, 0·25, 2·5 or 10 g/100 ml for 16 wk. No adverse effects on growth, organ weights, haematology, urine analysis or serum transaminase and alkaline-phosphatase activities were observed at the three lowest dose levels. Histological examination of tissues from animals at these dose levels revealed no abnormalities. On the other hand, at the highest dose level, male rats showed a decrease in weight gain and animals of both sexes showed skin lesions at the base of the tail, increased leucocyte counts, anaemia and an increase in the absolute and relative weights of the liver, kidneys and spleen. Histological changes in these animals were consistent with reticulo-endothelial activation in the liver and with vacuolar degeneration of the proximal convoluted tubules in the kidney.

The no-effect level of AHR-2438B in the drinking-water was found to be 2·5 g/100 ml, equivalent to a daily intake of 2·83 g/kg in males and 2·42 g/kg in females. These dose levels are approximately 50 times the estimated maximum intake for antipepsin activity in man.

## INTRODUCTION

AHR-2438B is the purified sodium salt of a lignosulphonate isolated from the waste sulphite liquor obtained as a by-product in the manufacture of paper pulp from Norwegian spruce. It is a low molecular weight lignosulphonate, the average molecular weight being approximately 5000. Its chemical structure, although not fully elucidated, can be considered to be that of a sulphonated polymer in which the basic unit is a propylbenzene structure similar to that of coniferyl alcohol.

Although sodium lignosulphonate has been used for many years as an animal-feed additive (Code of Federal Regulations, 1962), recent attention has been focused on the antipepsin property of lignosulphonates. In common with other lignosulphonates (Fletcher, Dahl, Jesseph, Steinbock & Harkins, 1957; Vocac & Alphin, 1968 & 1969), AHR-2438B is a competitive inhibitor of pepsin proteolysis and protects against the development of experimental gastric ulcers when it is administered orally to pyloric-ligated rats. This report presents the results of acute and subacute toxicity studies carried out in rats as part of a programme concerned with the evaluation of the relative safety of AHR-2438B.

## EXPERIMENTAL

*Test material.* AHR-2438B, a purified sodium lignosulphonate with a molecular weight of approximately 5000, was supplied by A. H. Robins Co. Ltd., Horsham, Sussex.

*Animals*. Male and female albino rats of the Wistar strain were purchased from A. Tuck & Son, Essex, England. Oxoid diet 41B and drinking-water were given *ad lib*.

*Acute toxicity*. Single doses of AHR-2438B were administered to groups of six male and six female rats by oral intubation of a 40% w/v solution in water. The animals (body weight 80–100 g) were fasted for 24 hr before dosing and were observed for 14 days after treatment for signs of toxicity. Autopsies were carried out on selected animals at day 14.

*Subacute toxicity*. Groups of 20 male and 20 female rats were housed five to a cage (with wire-mesh floor) in a room at controlled temperature and humidity. Four such groups were given AHR-2438B in the drinking-water in concentrations of 0·025, 0·25, 2·5 and 10 g/100 ml. A control group received untreated drinking-water. Administration of the test material was started after an initial conditioning period of 3 wk and continued for a further period of 16 wk. Body weight was recorded weekly and food and water consumption daily. From the overall mean daily fluid intake, the mean daily dose of AHR-2438B administered to each treatment group was calculated to be 0·017, 0·168, 2·83 and 10·02 g/kg in males and 0·026, 0·283, 2·42 and 9·99 g/kg in females for the groups given 0·025, 0·25, 2·5 and 10·0 g/100 ml drinking-water respectively.

Haematological investigations were carried out prior to treatment and during wk 6, 10 and 16. Blood was collected from the tail vein of groups of five animals and haemoglobin and haematocrit determinations, total erythrocyte and total and differential white cell counts and coagulation tests were carried out. In addition, blood samples were collected by cardiac puncture from rats under light ether anaesthesia for the determination of blood glucose, activities of serum glutamic–pyruvic and glutamic–oxalacetic transaminases and of serum alkaline phosphatase, serum urea and total serum protein content. Both control and treated animals were examined in wk 6, 10 and 16 for the presence of faecal occult blood, using the Hematest method.

Urine samples were collected in wk 6, 10 and 16 and tested for protein, ketone, glucose, blood and bile content. Spun-urine deposits were examined microscopically for epithelial cells, erythrocytes, mononuclear and polynuclear leucocytes, casts and other abnormal constituents.

The subacute toxicity test was terminated at the end of wk 16 when groups of ten animals from each of the treatment and control groups were killed by carbon dioxide euthanasia. The remaining animals were maintained on the established dosing schedules for further studies. At autopsy (wk 16) an examination was made for any gross abnormalities and the brain, pituitary, thyroid, spleen, heart, liver, kidneys, adrenals and sex organs were removed and weighed. Samples of these organs and of the pancreas, salivary gland, lung, thymus, oesophagus, stomach, duodenum, ileum, caecum, colon, rectum, lymph nodes and urinary bladder were preserved in 4% formaldehyde in normal saline and processed in the usual way for paraffin embedding. Sections of these tissues were stained with haematoxylin and eosin for microscopic examination.

## RESULTS

*Acute toxicity*

Single oral doses of up to 40 g AHR-2438B/kg were without effect on rats previously fasted for 24 hr except that diarrhoea occurred in animals receiving dose levels of 10 g/kg or more. At autopsy, no macroscopic or microscopic abnormalities were seen. Oral $LD_{50}$ values could not be determined because of the low toxicity of AHR-2438B.

*Subacute toxicity*

There were no differences from the controls in the general appearance or behaviour of rats treated with the three lowest dose levels of AHR-2438B. At the highest dose level, three male rats developed skin lesions at the base of the tail during wk 2 of treatment. Similar lesions developed in subsequent weeks in four more male and three female rats (Table 1). In addition to tail lesions, four male rats developed lesions of the scrotal sac. Animals presenting lesions showed signs of illness such as a reduction in body weight,

Table 1. *Development of tail lesions in rats receiving the highest dose level of AHR-2438B (10 g/100 ml drinking-water)*

| Sex | No. of rats/group | Total no. of animals with tail lesions at wk | | | | |
|---|---|---|---|---|---|---|
| | | 2 | 6 | 10 | 11 | 16 |
| Male | 20 | 3 | 5 | 6 | 6 | 7 |
| Female | 20 | 0 | 0 | 1 | 2 | 3 |

decreased locomotor activity and anaemia. Faeces of animals in the group on the highest dose were very soft, sticky and dark and were not segmented. The faecal changes became apparent between days 3 and 20 of treatment and continued throughout the period of administration. Severe diarrhoea was observed in two male rats, the stools being fluid in each case. With the three lowest dose levels of AHR-2438B, faecal consistency and colour were virtually unchanged, although in a few animals receiving 2·5 g/100 ml, the faeces were slightly softer and darker than those of controls. Despite the development of skin lesions in some animals, no faecal occult blood was detected in treated rats and in the terminal study no evidence of haemorrhage was found in the gastro-intestinal tract by low-power microscopical examination and histology.

Rates of growth were similar in treated and control animals with the exception of male rats receiving the highest dose of AHR-2438B (Table 2). The growth rate of this group of animals was significantly lower than that observed in other groups of male rats, from wk 6 onwards. Although female rats on the highest dose level showed a trend towards a reduced growth rate from wk 9 onwards, their weights did not differ significantly ($P > 0{\cdot}05$) from those of other female groups. Food and water consumption were similar in control and treated animals at each dose level throughout the test period (Table 3).

Haematological studies showed that only in the male rats receiving the highest dose level of AHR-2438B were there any consistent changes in the blood picture (Table 4). These changes were observed throughout the period of administration and consisted of reduced erythrocyte counts, reduced haemoglobin and haematocrit values and increased leucocyte counts. They appeared to be associated with the presence of lesions since rats that had not developed lesions possessed a blood picture similar to that of controls. On the other hand, rats with lesions presented marked anaemia with an increase in total leucocyte count. This is illustrated in Table 5, where haematological data are given for male rats both with and

Table 2. *Mean body weight of rats treated for 16 wk with AHR-2438B in the drinking-water*

| Dose level (g/100 ml) | Mean body weight (g) at wk | | | | | | Mean body weight gain (g) over 16 wk |
|---|---|---|---|---|---|---|---|
| | 0† | 3 | 6 | 9 | 12 | 16 | |
| **Males** | | | | | | | |
| 0·0 | 218 | 276 | 328 | 380 | 404 | 421 | 203 |
| 0·025 | 258** | 270 | 352 | 409 | 429 | 439 | 181 |
| 0·25 | 229 | 300 | 347 | 384 | 408 | 432 | 203 |
| 2·5 | 235 | 297 | 348 | 377 | 409 | 426 | 191 |
| 10·0 | 222 | 252 | 287*** | 318*** | 341*** | 373* | 151 |
| **Females** | | | | | | | |
| 0·0 | 187 | 216 | 224 | 241 | 241 | 243 | 56 |
| 0·025 | 198 | 230 | 240 | 248 | 250 | 253 | 55 |
| 0·25 | 189 | 221 | 232 | 245 | 241 | 249 | 60 |
| 2·5 | 190 | 221 | 232 | 243 | 243 | 250 | 60 |
| 10·0 | 190 | 213 | 225 | 237 | 234 | 233 | 43 |

†Day 1 of treatment.
Body weights are the means for groups of 20 rats and values marked with asterisks differ significantly (Student's *t* test) from those of controls: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

Table 3. *Mean values of food and water consumption of rats treated for 16 wk with AHR-2438B in the drinking-water*

| Dose level (g/100 ml) | Mean daily food consumption (g/rat) at wk | | | | | | Mean daily food consumption (g/rat) over 16 wk | Mean daily water consumption (ml/rat) at wk | | | | | | Mean daily water consumption (ml/rat) over 16 wk |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0† | 3 | 6 | 9 | 12 | 16 | | 0† | 3 | 6 | 9 | 12 | 16 | |
| **Males** | | | | | | | | | | | | | | |
| 0·0 | 19·6 | 30·9 | 29·3 | 29·3 | 29·3 | 29·4 | 27·6 | 25·3 | 27·3 | 31·7 | 27·0 | 28·0 | 23·9 | 28·6 |
| 0·025 | 22·5 | 35·3 | 30·7 | 29·4 | 26·6 | 28·6 | 27·7 | 26·2 | 28·1 | 30·1 | 27·9 | 23·9 | 24·3 | 27·0 |
| 0·25 | 20·7 | 32·3 | 30·7 | 29·4 | 25·7 | 30·9 | 27·4 | 24·8 | 25·0 | 26·3 | 28·3 | 27·6 | 24·4 | 25·7 |
| 2·5 | 21·9 | 32·4 | 30·7 | 32·3 | 28·0 | 33·4 | 28·7 | 23·6 | 27·0 | 26·4 | 25·0 | 24·7 | 24·6 | 26·0 |
| 10·0 | 23·1 | 30·1 | 30·6 | 30·7 | 28·9 | 29·1 | 28·6 | 24·3 | 29·7 | 31·7 | 30·9 | 33·3 | 25·0 | 31·9 |
| **Females** | | | | | | | | | | | | | | |
| 0·0 | 22·0 | 21·6 | 20·0 | 20·6 | 20·0 | 20·3 | 21·3 | 25·7 | 28·6 | 28·4 | 26·4 | 22·4 | 22·0 | 27·6 |
| 0·025 | 20·6 | 24·7 | 22·0 | 23·9 | 20·1 | 20·3 | 21·7 | 24·3 | 27·6 | 28·6 | 27·1 | 21·7 | 23·2 | 25·7 |
| 0·25 | 18·0 | 24·3 | 22·7 | 20·6 | 18·3 | 20·9 | 21·0 | 26·1 | 28·6 | 30·0 | 26·3 | 24·7 | 25·8 | 26·9 |
| 2·5 | 20·0 | 22·7 | 21·6 | 20·6 | 19·0 | 19·7 | 20·7 | 21·3 | 22·9 | 24·3 | 23·1 | 23·3 | 20·8 | 23·1 |
| 10·0 | 22·3 | 23·1 | 21·6 | 19·3 | 18·3 | 19·0 | 20·6 | 22·7 | 22·6 | 23·3 | 21·7 | 23·9 | 19·7 | 23·0 |

†Day 1 of treatment.

without lesions after the first 6 wk of the test period. Animals given lower dose levels showed no consistent changes in blood picture, although at wk 16, haemoglobin levels were reduced in male rats receiving 0·25 and 2·5 g/ml. However, it should be pointed out that after a

Table 4. *Haematological findings in rats treated for 6, 10 and 16 wk with AHR-2438B in the drinking-water*

| Dose level (g/100 ml) | RBC ($10^6$/mm$^3$) at wk | | | Hb (g/100 ml) at wk | | | Haematocrit (%) at wk | | | Total leucocytes ($10^3$/mm$^3$) at wk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 | 10 | 16 | 6 | 10 | 16 | 6 | 10 | 16 | 6 | 10 | 16 |
| **Males** | | | | | | | | | | | | |
| 0·0 | 8·00 | 7·97 | 8·29 | 13·3 | 14·5 | 14·9 | 45 | 48 | 49 | 11·6 | 12·1 | 8·8 |
| 0·025 | — | 8·18 | 3·12 | — | 14·5 | 14·7 | — | 47 | 47 | — | 7·4 | 6·5 |
| 0·25 | — | 8·03 | 8·18 | — | 14·0 | 13·4** | — | 46 | 44 | — | 21·1*** | 11·0 |
| 2·5 | 8·13 | 7·91 | 8·19 | 13·4 | 14·0 | 13·5** | 46 | 44 | 42** | 15·9 | 16·1 | 11·0 |
| 10·0 | 5·88* | 6·93* | 7·91 | 8·1** | 11·6* | 12·2*** | 35* | 39** | 42** | 27·1* | 21·7*** | 16·2* |
| **Females** | | | | | | | | | | | | |
| 0·0 | 7·79 | 7·96 | 7·62 | 12·5 | 14·1 | 14·0 | 43 | 45 | 45 | 7·3 | 5·8 | 5·0 |
| 0·025 | — | 7·64 | 7·77 | — | 14·1 | 13·9 | — | 46 | 45 | — | 7·6 | 5·6 |
| 0·25 | — | 7·95 | 7·62 | — | 14·2 | 13·9 | — | 46 | 40** | — | 7·9 | 4·3 |
| 2·5 | 8·16 | 7·89 | 7·66 | 13·1 | 13·7 | 14·9 | 46 | 44 | 45 | 10·6 | 9·3 | 8·5 |
| 10·0 | 7·92 | 7·70 | 7·02 | 10·9 | 12·6* | 12·7 | 38 | 42 | 42 | 13·8 | 11·2 | 10·4 |

RBC = Red blood cells Hb = Haemoglobin

Values are the means of groups of five animals and those marked with asterisks differ significantly (Student's *t* test) from control values: *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

further 2 wk of treatment, the haemoglobin levels of these rats had returned to normal. These changes were not therefore considered to be caused by AHR-2438B. Blood coagulation times were unaffected by the treatment.

No significant differences ($P > 0·05$) were observed in the levels of blood glucose, serum urea and serum protein in the treated and control rats (Table 6). Likewise, the serum levels

Table 5. *Haematology of five male rats with lesions and two male rats with no lesions after treatment for 6 wk with the highest dose level of AHR-2438B*

| Rat coding | Body weight (g) | RBC ($10^6$/mm$^3$) | Hb (g/100 ml) | Haematocrit (%) | Total leucocytes ($10^3$/mm$^3$) |
|---|---|---|---|---|---|
| **Rats with lesions** | | | | | |
| E 1 B | 229 | 6·472 | 9·0 | 37 | 19·2 |
| E 2 2L | 282 | 6·552 | 10·3 | 40 | 18·6 |
| E 3 2R | 186 | 2·728 | 3·7 | 21 | 25·2 |
| E 4 2R | 256 | 7·664 | 10·2 | 42 | 17·6 |
| E 4 1L | 315 | 6·936 | 8·1 | 35 | 16·6 |
| Means | 254 | 6·070 | 8·3 | 35 | 19·4 |
| **Rats with no lesions** | | | | | |
| E 3 B | 316 | 7·976 | 9·8 | 41 | 11·8 |
| E 3 1L | 306 | 7·744 | 11·4 | 43 | 12·2 |
| Means | 311 | 7·860 | 10·6 | 42 | 12·0 |

RBC = Red blood cells Hb = Haemoglobin

of glutamic–oxalacetic transaminase, glutamic–pyruvic transaminase and alkaline phosphatase were similar at each dose level. Chemical analyses indicated the absence of any abnormal constituent from the urine of all animals.

At autopsy, all animals in the highest dose group and some in the group given 2·5 g/100 ml showed congested mesenteric lymph nodes. In addition, significant increases in the absolute and relative weights of the liver, spleen and kidneys were found in the males and females which had received the highest dose level (Table 7). Although the weights of these organs in the groups given lower doses showed some dose-dependent increase in absolute and relative weights, these weights were not significantly different ($P > 0{\cdot}05$) from control values. Treatment with AHR-2438B did not affect the weights of other organs in animals of either sex.

Histological studies revealed that treatment with AHR-2438B at the three lowest dose levels induced no changes in any of the tissues examined. At the highest dose level, however, histological changes were observed in the liver and kidneys of both sexes. The livers showed normal parenchymal cells but there were marked increases in the size and number of reticulo-endothelial and Kupffer cells. The kidney showed swelling and vacuolation of the epithelial cells of the proximal convoluted tubules in the outer cortex, but were otherwise normal.

## DISCUSSION

Daily oral administration of AHR-2438B to rats did not alter growth rates except in males given the highest dose level, in which a significant retardation of weight gain occurred. A similar trend was observed in female rats on this highest dose level although the growth rates did not differ significantly ($P > 0{\cdot}05$) from those of controls. The reduced mean growth rate in males was not related to a lowered food intake but appeared to be related to the number of rats in the group with ulcerated lesions at the base of the tail. It can be seen from Table 5 that animals presenting tail lesions weighed less than unaffected animals and therefore the larger the proportion of affected animals in a group, the greater became the deviation from the mean body weight of unaffected animals. Thus from wk 6 onwards the number of males with lesions was sufficient to reduce the mean body weight of this group of rats to a level significantly different from control values. On the other hand, among the female rats of this group, the proportion of affected animals was smaller than in the corresponding group of males so that the mean body weight was not reduced to a level significantly different from that of the controls. In addition to the influence of tail lesions on growth rates, it is possible that weight gain may have been depressed to a limited extent as a result of a reduction in body fluid levels brought about by the continuous purgative action of very large doses of AHR-2438B.

The development of lesions in some animals appeared to be associated with the degree of adherence of sticky faeces to the affected areas. Lesions appeared in males earlier than in females since the scrotal sac trapped faeces between the base of the tail and the anus, and this aggravated the condition. The degree of faecal contamination varied from animal to animal and only in cases where the anal regions were continually very dirty did lesions develop. This suggested that the development of eroded areas occurred as a result of local irritation by adhering sticky faecal matter containing large quantities of the test material. It is possible that irritation was intensified by a wetting of the faeces with urine. The probability that lesions developed in this manner was supported by the fact that when five male

Table 6. *Blood and serum analyses of groups of five rats treated for 6, 10 and 16 wk with AHR-2438B in the drinking-water*

| Dose level (g/100 ml) | Blood glucose (mg/100 ml) at wk | | | Serum protein (g/100 ml) at wk | | | Serum urea (mg/100 ml) at wk | | | GOT (IU) at wk | | | GPT (IU) at wk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 | 10 | 16 | 6 | 10 | 16 | 6 | 10 | 16 | 6 | 10 | 16 | 6 | 10 | 16 |
| **Males** | | | | | | | | | | | | | | | |
| 0·0 | 87·4 | 108·9 | 100·3 | 6·2 | 6·5 | 6·5 | 15·9 | 17·6 | 16·3 | 44·3 | 65·4 | 39·9 | 8·7 | 12·7 | 12·2 |
| 2·5 | 91·8 | 114·3 | 89·4 | 5·9 | 6·5 | 6·5 | 15·6 | 19·3 | 14·2 | 42·6 | 54·9 | 39·9 | 5·1 | 7·1 | 11·9 |
| 10·0 | 97·4 | 110·8 | 97·4 | 5·2 | 5·9 | 6·1 | 21·7 | 23·7 | 20·2 | 45·2 | 63·2 | 33·9 | 10·8 | 14·3 | 6·7 |
| **Females** | | | | | | | | | | | | | | | |
| 0·0 | 86·8 | 107·0 | 86·5 | 5·9 | 6·7 | 7·0 | 17·3 | 16·1 | 19·5 | 50·5 | 59·7 | 40·9 | 11·5 | 8·1 | 7·2 |
| 2·5 | 87·6 | 107·8 | 90·1 | 5·5 | 6·4 | 6·9 | 22·7 | 22·7 | 22·0 | 48·0 | 54·1 | 25·9 | 5·5 | 6·3 | 1·9 |
| 10·0 | 100·0 | 114·8 | 100·7 | 5·4 | 5·9 | 6·4 | 19·7 | 20·8 | 18·1 | 44·2 | 55·5 | 35·4 | 6·4 | 8·1 | 1·6 |

GOT = Glutamic–oxalacetic transaminase GPT = Glutamic–pyruvic transaminase

Table 7. *Absolute and relative organ weights of rats treated for 16 wk with AHR-2438B in the drinking-water*

| Sex | Dose level (g/100 ml) | Terminal body weight (g) | Brain | Heart | Liver | Spleen | Kidneys | Adrenals† | Pituitary† | Thyroid† | Testes or uterus | Seminal vesicles or ovaries |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Absolute organ weight (g)** | | | | | | | | | | | | |
| Male | 0·0 | 421 | 1·89 | 1·20 | 16·65 | 0·79 | 3·44 | 53·0 | 21·2 | 20·6 | 3·17 | 1·32 |
| | 0·025 | 439 | 1·91 | 1·20 | 17·39 | 0·67 | 3·07 | 47·4 | 43·3 | 20·2 | 3·68 | 1·38 |
| | 0·25 | 431 | 1·85 | 1·35 | 17·23 | 0·81 | 3·48 | 48·8 | 46·2 | 19·0 | 3·19 | 1·53 |
| | 2·5 | 426 | 1·86 | 1·27 | 19·22 | 0·92 | 3·89 | 48·1 | 22·4 | 23·9 | 3·60 | 1·62 |
| | 10·0 | 372 | 1·84 | 1·16 | 20·04** | 0·97** | 4·11** | 57·4 | 35·0 | 17·8 | 3·98 | 1·36 |
| Female | 0·0 | 243 | 1·83 | 0·85 | 10·41 | 0·58 | 2·10 | 75·8 | 25·1 | 14·5 | 0·53 | 0·12 |
| | 0·025 | 253 | 1·81 | 0·78 | 9·97 | 0·50 | 1·77 | 63·5 | 38·7 | 13·1 | 0·58 | 0·11 |
| | 0·25 | 249 | 1·76 | 0·80 | 9·75 | 0·49 | 1·97 | 68·8 | 44·1 | 16·0 | 0·55 | 0·11 |
| | 2·5 | 250 | 1·82 | 0·77 | 10·48 | 0·56 | 2·19 | 69·2 | 32·5 | 12·5 | 0·48 | 0·11 |
| | 10·0 | 233 | 1·83 | 0·73 | 12·17** | 0·70** | 2·84** | 89·0 | 26·3 | 16·3 | 0·46 | 0·10 |
| **Relative organ weight (g/100 g body weight)** | | | | | | | | | | | | |
| Male | 0·0 | | 0·45 | 0·29 | 3·98 | 0·19 | 0·83 | 0·012 | 0·005 | 0·004 | 0·76 | 0·31 |
| | 0·025 | | 0·42 | 0·26 | 3·80 | 0·15 | 0·67 | 0·010 | 0·009 | 0·004 | 0·80 | 0·30 |
| | 0·25 | | 0·43 | 0·32 | 4·05 | 0·19 | 0·82 | 0·011 | 0·010 | 0·004 | 0·75 | 0·36 |
| | 2·5 | | 0·43 | 0·29 | 4·41 | 0·21 | 0·89 | 0·011 | 0·005 | 0·005 | 0·83 | 0·37 |
| | 10·0 | | 0·48 | 0·30 | 5·19** | 0·25** | 1·06** | 0·014 | 0·009 | 0·004 | 1·01 | 0·35 |
| Female | 0·0 | | 0·70 | 0·32 | 3·96 | 0·22 | 0·80 | 0·028 | 0·009 | 0·005 | 0·20 | 0·05 |
| | 0·025 | | 0·71 | 0·31 | 3·93 | 0·20 | 0·70 | 0·024 | 0·014 | 0·005 | 0·23 | 0·04 |
| | 0·25 | | 0·70 | 0·32 | 3·87 | 0·19 | 0·78 | 0·026 | 0·017 | 0·006 | 0·22 | 0·04 |
| | 2·5 | | 0·71 | 0·30 | 4·06 | 0·23 | 0·87 | 0·026 | 0·012 | 0·004 | 0·18 | 0·04 |
| | 10·0 | | 0·81 | 0·32 | 5·38** | 0·31** | 1·26** | 0·039 | 0·011 | 0·007 | 0·20 | 0·05 |

†Absolute weights of this organ are expressed in mg.
Values are the means for groups of ten rats. Those marked with asterisks differ significantly (Student's *t* test) from the control values: $^{**}P < 0{\cdot}01$.

and two female rats with lesions were placed in cages containing sawdust and cleansed twice daily in the anal region, the lesions healed or had substantially regressed within 5 wk of separation despite continued lignosulphonate treatment. At the same time an improvement in the blood picture was observed, suggesting that the anaemia and raised leucocyte levels in these animals was a result of the presence of the tail lesions.

It has recently been reported by Marcus & Watt (1969) that oral administration of carrageenans produces ulcerative lesions in the caecum, colon and rectum of some animal species, including the rat. Since AHR-2438B produced external ulcerative areas at the highest dose level and showed certain other similarities to the carrageenans, it was important in this study to determine whether AHR-2438B also caused ulcerative lesions of the rat intestinal mucosa. In fact, no faecal occult blood was found in animals receiving this material and since this is an early and reliable indication of the onset of ulcerative colitis, it was concluded that AHR-2438B does not produce such lesions in the rat. This was confirmed at autopsy, when no ulcerative lesions were found in any section of the gastro-intestinal tract.

It is well documented that sulphonated macromolecules such as sulphonated polysaccharides possess a powerful heparin-like anticoagulant property. Thus, it might be expected that AHR-2438B would also possess this property. However, this is not the case, since even at the highest dose level of AHR-2438B, blood coagulation times were unchanged throughout the period of administration. Furthermore, *in vitro* studies have indicated that the drug does not prolong blood-coagulation time in the rat at a concentration of 2·5 mg/ml. The explanation for this is no doubt connected with the molecular weight of AHR-2438B, which is approximately 5000. For example, Loomis & Beyer (1953) showed that the anticoagulant activity of sulphonated lignins was related to their molecular weight, high molecular weight fractions possessing greater anticoagulant activity than the lower molecular weight fractions. In addition, Vocac & Alphin (1968 & 1969) have reported that several of the low molecular weight lignosulphonates that they examined possessed very little heparin-like anticoagulant activity.

Biochemical and histological examination of the kidney and liver revealed no toxic effects induced by continuous treatment with AHR-2438B at the three lowest dose levels. However, histological findings in the group given the highest dose suggested that the material was absorbed and stored to some extent. For example, swelling and vacuolation of the epithelium of the proximal convoluted tubules of the kidney was consistent with the storage of material and so was the marked increase in the size and number of reticulo-endothelial and Kupffer cells in the liver. Likewise, the increase in weight of these organs compared with the controls at autopsy may indicate storage of AHR-2438B, since Golberg (1966) has reported that liver enlargement occurs following the administration of other macromolecular compounds, such as iron-dextran and methylcellulose.

The results of the acute oral toxicity tests indicate that AHR-2438B has a low acute toxicity, since it was impossible to produce death in rats by a single oral dose of up to 40 g/kg. The relative safety of the material is further supported by the lack of toxicity shown in the subacute toxicity tests. This study shows that the no-effect level of AHR-2438B administered daily in the drinking-water of rats for 16 wk is 2·5 g/100 ml, which is equivalent to a daily intake of 2·83 g/kg in males and 2·42 g/kg in females. These dose levels are approximately 50 times the estimated maximum intake by man.

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### Toxicité orale aiguë et subaiguë d'un lignosulfonate de sodium purifié, l'AHR-2438B, chez le rat

**Résumé**—La $DL_{50}$ orale aiguë de l'AHR-2438B est de > 40 g/kg chez le rat.

Pour déterminer la toxicité subaiguë du produit, on en a ajouté pendant 16 semaines 0 (témoins), 0,025, 0,25, 2,5 ou 10 g/100 ml à l'eau de boisson d'un groupe de rats. On n'a pas observé d'effets nuisibles à la croissance, aux poids des organes, à la composition du sang et de l'urine et aux activités de la transaminase et de la phosphatase alcaline sériques chez les animaux qui recevaient les trois doses inférieures et l'examen histologique des tissus de ces animaux n'a révélé aucune anomalie. Dans le groupe qui recevait la plus forte dose, par contre, on a constaté chez les mâles une diminution du gain de poids et chez les animaux des deux sexes des lésions cutanées à la base de la queue, des augmentations du nombre de leucocytes, de l'anémie et une augmentation des poids absolus et relatifs du foie, des reins et de la rate. Les modifications histologiques observées chez ces animaux concordaient avec une activation réticulo-endothéliale du foie et une dégénérescence vacuolaire des tubes contournés proximaux du rein.

Le seuil d'indifférence de l'AHR-2438B dans l'eau de boisson, déterminé à 2,5 g/100 ml; équivaut à une consommation journalière de 2,83 g/kg chez les mâles et de 2,42 g/kg chez les femelles. Ces taux correspondent à environ 50 fois la consommation maximale estimée nécessaire pour une activité antipepsique chez l'homme.

### Akute und subakute orale Toxizität von AHR-2438B, einem gereinigten Lignosulfonat, in Ratten

**Zusammenfassung**—Die akute orale $LD_{50}$ von AHR-2438B für Ratten wurde mit > 40 g/kg bestimmt.

Bei subakuten Toxizitätsversuchen erhielten Ratten AHR-2438B 16 Wochen lang im Trinkwasser in Konzentrationen von 0 (Kontrolle), 0,025, 0,25, 2,5 oder 10 g/100 ml. Bei den drei niedrigsten Dosierungen wurden keine nachteiligen Wirkungen auf Wachstum, Organgewichte, Hämatologie, Urinzusammensetzung und Serumtransaminase- und alkalische Phosphataseaktivität festgestellt. Die histologische Untersuchung von Geweben der Tiere, welche diese Dosierungen erhielten, zeigte keine Anomalien. Andererseits zeigten bei der höchsten Dosierung männliche Tiere eine Verminderung der Gewichtszunahme und Tiere beider Geschlechter Hautläsionen an der Schwanzwurzel, erhöhte Leukozytenzahlen, Anämie und eine Zunahme des absoluten und des relativen Gewichts von Leber, Nieren und Milz. Die histologischen Veränderungen bei diesen Tieren stimmten mit den Erscheinungen reticuloendothelialer Aktivierung in der Leber und mit vacuolärer Degeneration der proximalen convoluten Nierenkanälchen überein.

Die wirkungsfreie Konzentration von AHR-2438B im Trinkwasser wurde mit 2,5 g/100 ml bestimmt, was einer täglichen Aufnahme von 2,83 g/kg durch männliche und von 2,42 g/kg durch weibliche Tiere entspricht. Diese Konzentrationen sind etwa das 50fache der geschätzten maximalen Aufnahme an Antipepsinaktivität beim Menschen.