KCl solution which simulated the $[K^+]$ concentration of the seed extract (1.15 M) was treated similarly. (The $[K^+]$ of solutions following such treatment was $< 1$ mM.) Solutions of $\alpha$- and $\beta$-ODAP (from *L. sativus* seed[12]), and $MgCl_2$ and $MnCl_2$ were prepared in Ringer solution, to simulate the concentrations of these substances in the diluted seed extract. A sample of seed extract was ashed to remove organic material and diluted as before with Ringer solution. Each solution was tested on at least 3 different nerve preparations (table 2).

*Discussion.* The results suggest that the high $K^+$ content of the *L. sativus* seed extract was the major cause of the abolition of the action potential in frog sciatic nerve, but do not exclude the possibility that sodium cobaltinitrite removed a neurotoxic substance as well as $K^+$. This is unlikely as similar results were obtained when $K^+$ was removed with sodium perchlorate (results not shown). The activity of the ashed extract excludes a synergistic action of an organic neurotoxin with $K^+$. The results also exclude the direct involvement of $Mn^{2+}$ (Sadasivan et al.[13]) and $Mg^{2+}$, of $\alpha$- and $\beta$-ODAP, and presumably the other known neurotoxins in *L. sativus* seed. Although we have confirmed the previously reported findings[8], no evidence was obtained that *L. sativus* seed contains a neurotoxin which effects the activity of amphibian peripheral nerve in vitro following topical application. Our own and previous findings therefore are probably solely attributable to the effects of the high $K^+$ content of the seed extract.

Norris et al.[8] also reported that an i.v. bolus of 10–15 ml of *L. sativus* seed extract (approximately 1.8 M $K^+$ from our calculations) caused electrocardiographic changes, bradycardia, a fall in blood pressure and death in dogs. In monkeys an i.v. bolus of 15–20 ml of crude *L. sativus* seed extract (approximately 0.5 M $K^+$ from our calculations) was reported to cause stupor, coma and death[6]. The established effects of a high circulating $[K^+]$ on cardiac function[14] makes it likely that these effects too were mediated mainly by the the very large amounts of $K^+$ which the seed extracts contained.

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2 H. Selye, Revue can. Biol. *16*, 1 (1957).
3 M.V. Barrow, C.F. Simpson and E.J. Miller, Q. Rev. Biol. *49*, 101 (1974).
4 S.L.N. Rao, P.R. Adiga and P.S. Sarma, Biochemistry *3*, 432 (1964).
5 C. Rukmini, Ind. J. óhem. *7*, 1062 (1969).
6 V. Nagarajan, V.S. Mohan and C. Gopalan, Ind. J. Med. Res. *53*, 269 (1965).
7 S.L.N. Rao, J. Neurochem. *30*, 1467 (1978).
8 B. Norris, J. Concha and C. Reyes, Boln Soc. Biol. Concepción *37*, 29 (1962).
9 E.A. Bell and J.P. O'Donovan, Phytochemistry *5*, 1211 (1966).
10 J. Heilmann, J. Barrollier and E. Watzke, Z. Physiol. Chem. *309*, 219 (1957).
11 A.F. Huxley and R. Stämpfli, J. Physiol. *112*, 496 (1951).
12 F.L. Harrison, P.B. Nunn and R.R. Hill, Phytochemistry *16*, 1211 (1977).
13 T.S. Sadasivan, C.B. Sulochana, V.T. John, M.R. Subbaram and C. Gopalan, Curr. Sci. *29*, 86 (1960).
14 A.S.V. Burgen and K.G. Terroux, J. Physiol. *119*, 139 (1953).

## Possible mutagenic activity of saccharin

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*Summary.* A mutagenic effect of saccharin in Chinese hamsters, using the in vivo SCE test, was observed when massive overdoses were administered; cyclamate was not mutagenic.

Mutagenic effects of saccharin have been reported for several years. Some authors regard not the saccharin itself, but contaminants present in the preparations used, as responsible for such effects. According to a review of the National Academy of Sciences panel on saccharin, 21 mutagenicity studies on saccharin available until the end of 1978 yielded 16 negative and 5 positive results[1]. Carcinogenetic effects (carcinoma of the bladder in male rats[2]) can be regarded as being consistent with the positive mutagenicity findings. The following conclusions have been drawn from a compilation and evaluation of the results of 17 mutagenicity studies on saccharin up to 1975[3]:

Saccharin is a weak mutagen:
- at very high doses in *Salmonella;*
- at moderate doses in *Drosophila;*
- at medium to very high doses in mice.

Saccharin was found to have a low chromosome-breaking effect in plant roots and in Chinese hamster cells, but in these and other test systems doubtful and negative results were obtained as well. Altogether there is an indication that saccharin is capable of chromosome-breaking rather than of inducing point mutations.

A much more sensitive test than the chromosome aberration test for the detection of mutagenic substances is the sister chromatid exchange (SCE) test. After 2 cell cycles, the chromatids of mammalian chromosomes are stained differentially in most cells in the presence of 5-bromodeoxyuridine (BUdR), a base analogue of thymidine. SCEs appear as reciprocal exchanges of DNA between the chromatids. The number of SCEs increases depending on the mutagenic dose added. Using this test, a mutagenic effect of saccharin at relatively high concentrations was verified recently[4]; this study revealed also strongly different degrees of sensitivity between the cell types used (human lymphocytes and CHO cells), a phenomenon observed earlier in other test systems[5].

For 2 years, the SCE test has also been carried out as an in vivo test in small mammals[6,7], and since 1978 in an improved, standardized form[8]. Using this new test we examined the question of a possible mutagenic effect of saccharin. Chinese hamsters (10–11 weeks old, average weight 30 g) were implanted s.c. with 50-mg BUdR tablets, 2 h later they received sodium saccharin (pure quality C. Roth, D-7500 Karlsruhe) by stomach tube (40%, dissolved in water) and after 24 h 1 mg/kg colcemid. After 26 h the animals were sacrificed to obtain cell suspensions from the femur bone marrow[9]. The results are shown in the table.

Induction of SCEs by saccharin (oral administration of sodium saccharin, bone marrow cells of Chinese hamsters; 5 animals per dose; 100 cells per animal

| | |
|---|---|
| Control | $=3.82\pm0.15$ SCEs/cell |
| 1 g saccharin/kg b.wt. | $=3.84\pm0.20$ SCEs/cell |
| 5 g saccharin/kg b.wt. | $=5.16\pm0.17$ SCEs/cell |
| 7.5 g saccharin/kg b.wt. | $=5.70\pm0.25$ SCEs/cell |
| 10 g saccharin/kg b.wt. | $=6.61\pm0.81$ SCEs/cell |

On the basis of long-term toxicity studies an FAO/WHO expert committee[10] determined acceptable daily intake (ADI) values for saccharin of 5 mg/kg b.wt in general, and 5–15 mg/kg for diabetics. Acute $LD_{50}$ (oral administration, mice and rats) is about 17 g/kg. According to the table, doses of 7.5 g/kg had to be administered in the test animals in order to reach the 1.5 times increase of the 0-value for SCEs. This increase in the SCE rate was obvious, but one has to bear in mind that such doses are already in the toxic range; that they are equivalent to a daily intake of about 500 g of saccharin by humans, and that foods containing such massive overdoses of saccharin would be inedible. The same applies to carcinogenicity studies which yielded positive results. Obviously, the risk posed by the weak mutagenicity and weak carcinogenicity of saccharin has to be weighed against its benefits for diabetics and for the obese. In studies parallel to those described here we have also examined the sweetener cyclamate, as well as its metabolite cyclohexylamine, by means of the in-vivo SCE test. No indications of a mutagenic effect due to cyclamate were found even at the highest dose administered (sodium cyclamate, pure quality, 10 g/kg $=3.99\pm0.08$ SCEs/cell).


1 Food Chemical News, Nov. 20 (1978).
2 D.L. Arnold, C.A. Moodie, M.C. Grice, S.M. Charbonnean and I.C. Munro, Toxicology Research Division, Health Protection Branch, Ottawa, Canada, April 1977.
3 P.G.N. Kramers, Mut. Res. *32*, 81 (1975).
4 S. Wolff, B. Rodin, Science *200*, 543 (1978).
5 D.T. Arakaki and W. Schmid, Humangenetik *11*, 119 (1971).
6 W. Vogel and Th. Bauknecht, Nature *260*, 448 (1976).
7 J.W. Allen and S.A. Latt, Nature *260*, 449 (1976).
8 J.W. Allen, C.F. Shuler, R.W. Mendes and S.A. Latt, Cytogenet. Cell Genet. *18*, 231 (1977).
9 H.W. Renner, Drug Res., in press (1979).
10 FAO Nutrition Meetings Report Series No.44A, WHO/Food Add./68.33, 1967.


## 50 ppm MnBK subclinical neuropathy in rats[1]


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*Summary.* 40 rats were subjected daily for 6 months to an atmosphere containing 50 ppm MnBK. 32 of the rats presented with demyelination of the sciatic nerve and 2 of these with axonal hypertrophy.


In August 1973, Dr Mary A. Gillchrist, reported a patient with acute peripheral neuropathy who was an employee of the print department of a coated fabrics plant in Columbus, Ohio[3]. Several of the patient's co-workers also had peripheral neuropathy of recent origin. The use of methyl n-butyl-ketone (MnBK) in the plant[4] was suspected. Subsequently it was shown that MnBK causes a neuropathy in experimental animals[5–7]. The acute toxicity of MnBK had been examined in the past and no neurotoxicity reported[8]. Subsequent studies have elaborated these findings, described the clinical manifestations in humans and suggested that the neurotoxic effects of MnBK, N-hexane, and 2,5-hexadione may have a common metabolic origin[9–14]. The purpose of our studies was to find the lowest levels of toxicity of MnBK and to investigate the possible neurotoxicity of methyl isobutyl-ketone (MiBK) and methyl-ethyl-ketone (MEK).

*Materials and methods.* Wistar white rats were placed in 55-gallon metal drums, maintained under normal pressure, with ports to control the atmosphere. The levels of the various ketones in the air in the drum were verified with gas chromatography. 5 groups of 20 rats were exposed – 8 h per day, 5 days a week – to: 1. 50 ppm MiBK for 17 weeks. 2. 40 ppm MnBK for 22–88 days. 3. 50 ppm MnBK for 13 weeks. 4. 700 ppm MEK for 16 weeks. 5. 200 MEK for 17 weeks. Similar numbers of control rats were placed in the drums and exposed to an atmosphere free of ketones. There were no clinical nor pathological manifestations of a peripheral neuropathy in any of the experimental rats, except 3 of 20 rats exposed to 50 ppm MnBK for 13 weeks

Previous and present experiments with MnBK, MEK and MiBK

| | 1st experiment | | 2nd experiment | | | | | 3rd experiment |
|---|---|---|---|---|---|---|---|---|
| | MnBK | MnBK and MEK | MiBK | MnBK (w) | | MEK | | MnBK |
| | | | | 1 | 2 | 1 | 2 | |
| Dose (ppm) | 200 | 200 | 50 | 40 | 50 | 700 | 200 | 50 |
| Time | 6 w | 6 w | 17 w | 22–88 d | 13 w | 16 w | 17 w | 6 m |
| Number of rats | 9 | 8 | 20 | 20 | 20 | 20 | 20 | 40 |
| Clinical | + | ++ | – | – | – | – | – | – |
| patn/PNS | + | + | | | 3+ | | | + |
| CNS | – | – | – | – | – | – | – | + |

This table presents our previous and present experiments with MnBK, MEK and MiBK. The results of the 1st experiment, published previously[3,7], showed that 200 ppm MnBK or MnBK and MEK present daily in the atmosphere for 6 weeks, resulted in temporary paralysis and demyelination. The results of our 2nd experiment showed no clinical nor pathological changes, except for 3 rats which received 50 ppm for 13 weeks. The 3rd experiment, described in this report, showed that 32 of the rats presented with demyelination, and only 3 with axonal hypertrophy.