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]

S. Duckett[2], L.J. Streletz, R.A. Chambers, M. Auroux and P. Galle

Department of Neurology, Jefferson Medical College, Thomas Jefferson University, Philadelphia (Pa 19107, USA), and the Departments of Biophysics (Creteil) and Neurology (Kremlin-Bicêtre), University of Paris, Paris (France), 11 December 1978

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.

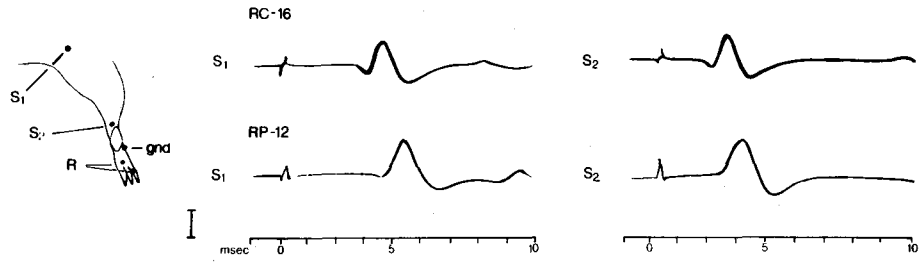


Fig. 1. Left: Diagram of recording technique. S_1 and S_2 stimulating proximal and distal cathodes; R: recording electrode; Gnd: ground electrode. Right: Muscle action potentials following distal and proximal stimulation in rats RC-16 (control) and RP-12 (50 ppm MnBK for 6 months). Conduction velocities were 44 and 35 m/sec, respectively.

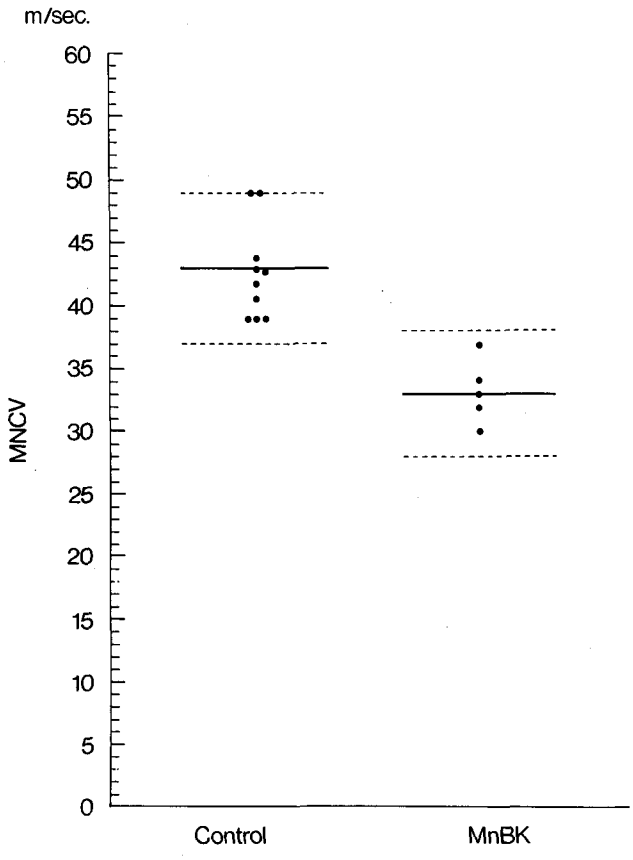


Fig. 2. Maximal nerve conduction velocity in motor fibres of sciatic nerve in control and MnBK intoxicated rats (50 ppm for 6 months). Solid lines indicate mean conduction velocities; dotted lines are 2 SD from the mean ($p = 0.005$).

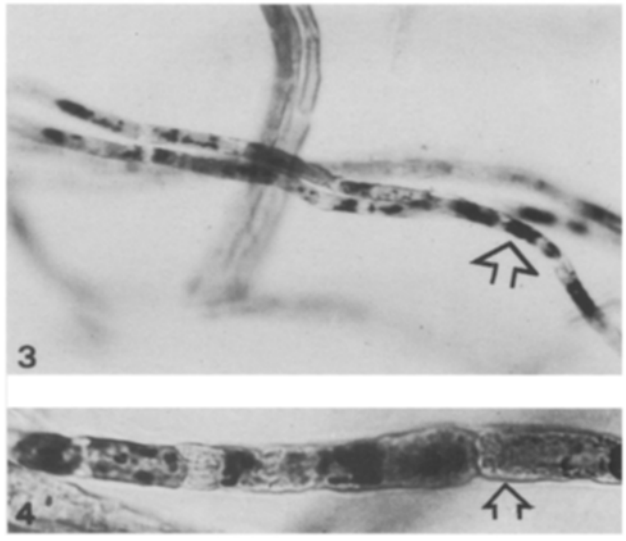


Fig. 3 and 4. Marchi positive degenerate myelin in individualized nerve fibres, indicated by arrows.

who presented with demyelination of the sciatic nerve (table). On the basis of this result we devised an experiment where 40 rats received 50 ppm MnBK for 6 months, 8 h a day, 5 days a week, in drums as described above. 20 control rats placed in drums received only fresh air without MnBK for a similar period. This is a preliminary report of our observations of this last experiment.

Electrophysiologic studies were performed on 5 of the exposed rats and 10 of the control animals chosen randomly at the end of the experiment. Motor nerve conduction velocity (MNCV) in the fibres supplying the small muscles of the plantar surface of the hind foot was studied in anesthetized animals according to the method described by Fullerton[15]. The rats were sacrificed with ether and perfused with cold 10% formalin, buffered with 0.1 M sodium phosphate, pH 7.2. The histological methods used to examine the tissues were hematoxylin-eosin, Holmes-silver for nerve fibres, Nauta-Gyggax for degenerative axons, Golgi preparations for nerve fibres, Marchi preparations for degenerative myelin were dissected in glycerin. Tissues from the CNS, liver and kidney were examined with light microscopy.

Results. During life the MnBK intoxicated rats were as active and normal as the controls. 2 estimations of maximal MNCV per animal were performed in 10 control animals. In one animal, the MNCV for the fastest fibres between hip and ankle was calculated as 44 m/sec (f 1). Conduction velocities for all control animals were plotted and ranged between 39 and 49 m/sec (SD ± 3.7) with a mean averaged velocity of 43 m/sec (f 2). In the MnBK exposed group 2 MNCVs were obtained in each of 5 animals after 17 weeks of exposure. Conduction velocities for the group ranged between 30 and 37 m/sec (SD ± 2.5). The mean for the MnBK group was 33 m/sec and in all animals the MNCV's were slower than the control group (f 1, 2). No significant difference in the amplitude of the muscle action potentials was evident in comparison to the control group. Histological examination of the sciatic nerve showed widespread demyelination with deposits of Marchi positive material diffusedly distributed in the nerve fibres of 32 of the rats examined. Axonal hypertrophy and beading were present in the sciatic nerves of 2 of these rats. The sciatic nerves of the control rats were normal. The CNS, liver and kidney of all the rats were normal.

Discussion. The extensive demyelination and minimal axonal changes described here are in apparent contradiction

to previous studies of MnBK-induced neuropathy which described axonal pathology with secondary demyelination (see review in Spencer and Schaumberg[13]) possibly because of the low qualities of MnBK used in this study. Low doses of a toxicant such as lead or colchicine can cause a segmental demyelination and high doses cause axonal degeneration or both[15-17]. Extensive segmental demyelination with some Wallerian degeneration has been reported in animals intoxicated with *Karwinskia humboldtiana* and in tellurium neuropathy[18,19].

1 This work was supported in part by General Support Grant RR. 5414.
2 We thank Dr Cedric Raines, Einstein Medical Center, New York, for helpful criticisms and suggestions and Dr Saul Francis for intoxicating the rats.

3 M.A. Gilchrist, W.E. Hunt, N. Allen, H.T. Yee and D.J. Billmaier, Morb. Mort. Wk Rep. *23*, 9 (1974).
4 D.J. Billmaier, H. Yee, N. Allen, N. Craft, S. Williams, S. Frostlin and R. Fontaine, J. occup. Med. *16*, 665 (1974).
5 S. Duckett, N. Williams, S. Francis, New Engl. J. Med. *290*, 1264 (1974).
6 J.R. McDonough, New Engl. J. Med. *290*, 1263 (1974).
7 J.R. Mendell, K. Saida and M.F. Ganansia, Science *185*, 787 (1974).
8 E. Browning, Toxicity and metabolism of industrial solvents, p.428. Amsterdam 1965.
9 S. Duckett, N. Williams and S. Francis, Experientia *30*, 1283 (1974).
10 M.S. Abdel-Rahman, L.B. Hetland and D. Couri, Am. Indian Hyg. Ass. J. *1976*, 95.
11 N. Allen, J.R. Mendell and D.J. Billmaier, Arch. Neurol. *32*, 209 (1975).
12 G.D. DiVencenzi, C.J. Kaplan and J. Dedinas, Toxic. appl. Pharmac. *36*, 511 (1976).
13 P.S. Spencer and H.H. Schaumberg, J. Neuropath. exp. Neurol. *36*, 300 (1977) (see review).
14 J.R. Mendell, New Engl. J. Med. *290*, 1263 (1974).
15 P.M. Fullerton, J. Neuropath. exp. Neurol. *25*, 214 (1966).
16 J.B. Angevine, J. exp. Zool. *136*, 363 (1957).
17 J.M. Jacobs, J.B. Cavanagh and F.C. Chen, J. Neurol. Sci. *17*, 461 (1972).
18 J. Mitchell, R.O. Weller, H. Evans, I. Arai and G.D. Davies, Neuropath. appl. Neurobiol. *4*, 85 (1978).
19 S. Duckett, G. Said, L.J. Streletz, R.G. White and P. Galle, Neuropath. appl. Neurobiol. *5*, 265 (1979).

The principal toxin of *Delphinium brownii* Rydb., and its mode of action

V. Nambi Aiyar, M.H. Benn*, T. Hanna, J. Jacyno, S.H. Roth and J.L. Wilkens[1]

Departments of Biology, Chemistry, and Pharmacology and Therapeutics, The University of Calgary, Calgary (Alberta, Canada T2N 1N4), 13 October 1978

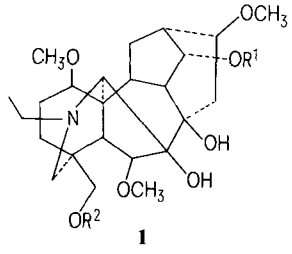
Summary. Examination of *D. brownii*, a stock-poison of Western Canada, revealed that the principal toxin was methyllycaconitine: a potent neuromuscular blocking agent which appears to act competitively at nicotinic receptors.

In North America, *Delphinium* are held responsible for more cattle deaths than any other kind of poisonous plant[2]. Although the problem is an old one, and the toxicity of the plants has long been attributed to the alkaloids which they contain, systematic studies of the toxins and their modes of action are lacking: a matter of substance in determining the best treatment for poisoned animals. We report here the results of a study of the toxins of *D. brownii* Rydb., an important stock-poison of the ranges of Western Canada.

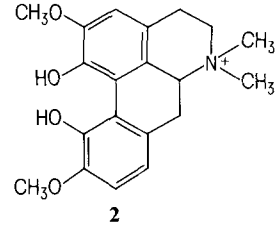
The leafy stems of *D. brownii* were collected in early (crown) stages of growth, and a methanolic extract of the plants was separated by conventional procedures[3] into non-alkaloidal, and alkaloidal fractions; the latter being subdivided into water-insoluble bases, and water-soluble quaternary ammonium salts. Further fractionation[3] of the bases yielded as the major constituents the tertiary diterpenoid alkaloids methyllycaconitine (MLA, **1a**), browniine **(1b)**, and browniine acetate **(1c)**. The principal component of the quaternary ammonium salts was the aporphine magnoflorine **(2)**, although this compound was only present in the plants in very minor amount.

Prominent among the symptoms of *D. brownii* poisoning is paralysis. Together with the published results of work in the Soviet Union on the pharmacology of some diterpenoid alkaloids[4-8], this suggested that a primary site of action of the toxins was the neuromuscular junction. Accordingly, in addition to preliminary acute toxicity tests on mice, the extracts were screened using 2 in vitro assays. Thus the depressant effects of the extracts were examined on the responses of the electrically-induced muscle twitch of the isolated rat phrenic nerve-diaphragm preparation[9], and an electrically stimulated longitudinal muscle strip of guinea-pig ileum[10]. Aconitine hydrochloride, and (+)-tubocurarine chloride hydrochloride were used as reference standards. Alkaloids were tested as water-soluble hydrochloride preparations, while water-insoluble extracts were added to organ baths as suspensions in dilute Cremophor EL (polyoxylated castor oil, BASF (Canada)) produced by sonication.

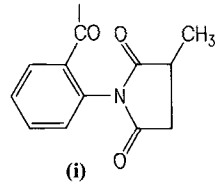
The toxicity of the plant extracts clearly resided in the tertiary base fraction. At the individual alkaloid level none of the compounds **1a–c**, or **2**, exhibited much muscarinic activity (as tested on the ileum preparation). However the crude tertiary, alkaloid fractions showed significant nicotinic blocking action (in the phrenic nerve-diaphragm system). Alkaloids **1b–c**, and **2** were of relatively low activity in this test, but **1a** was much more effective (see table), and as a



1



2



(i)

1a $R^1 = OCH_3$ $R^2 = (i)$
1b $R^1 = OH$ $R^2 = OCH_3$
1c $R^1 = OCOCH_3$ $R^2 = OCH_3$
1d $R^1 = OCH_3$ $R^2 = OH$