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Acute and Short-term Oral Toxicity of 2-*N*-Ethylaminoethanol in Rats

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Abstract—Acute oral doses of neutralized 2-*N*-ethylaminoethanol (EAE) in rats greatly increased gastric secretion, resulting in dehydration and death after 3–4 days. The effect of EAE on gastric secretion did not appear to be due to osmotic effects or to histopathological changes in the gastro-intestinal tract.

Short-term administration of neutralized EAE in the drinking water produced evidence of pronounced cumulative toxicity. The 30-day LC_{50} was 1412 ± 710 ppm. At lower concentrations given over a period of 90 days the main toxic effects of EAE were retardation of growth and degenerative changes in the liver and kidneys. A concentration of 25 ppm EAE in the drinking water for 90 days resulted in minor changes and was considered to be close to the no-effect level.

INTRODUCTION

2-*N*-Ethylaminoethanol (EAE) is a member of the group of *N*-substituted aminoethanols that have been widely used as emulsifiers, dispersants, gas absorbants, detergents, lubricants and chemical intermediates.

Smyth, Carpenter, Weil & Pozzani (1954) found an acute oral LD_{50} of 1·48 g/kg for the unneutralized material in rats. Cornish & Adefuin (1967) reported that neutralized EAE given in drinking water at 4000 ppm resulted in a considerable number of deaths at the end of 1 wk.

The toxicity of the closely related 2-*N*-diethylaminoethanol has been studied (Rosenberg, Kayden, Lief, Mark, Steele & Brodie, 1949; Kraatz, Gruber & Lisi, 1950), but similar detailed studies on the toxicity of EAE appear to be lacking. Since the vapour pressure of EAE is very low the most significant exposure to EAE is likely to be by ingestion. These studies were concerned, therefore, mainly with oral administration.

EXPERIMENTAL

Materials. EAE was purified by fractional vacuum distillation and neutralized with 12 N-HCl prior to use. All dosages are given in terms of EAE base.

Animals. Sprague–Dawley (Spartan strain) rats were used in both the acute and short-term studies.

Acute toxicity. Single doses of 0–2 g neutralized EAE/kg were given by oral intubation to groups of 20 male and 20 female rats (210–260 g body weight). Single intraperitoneal doses of 0–1·5 g/kg were given to groups of 20 male rats (260–300 g body weight). Rats, which

were not fasted before dosing, were observed for 14 days after treatment, after which autopsies were carried out. LD_{50} values were computed by the method of Berkson (1944). Gastric secretion was measured by gastric lavage using normal physiological saline under light pentobarbitone anaesthesia after pyloric ligation in 18 male rats, 3 days after a single oral dose of 1·5 g EAE/kg. Water absorption was determined indirectly by measuring the rate of appearance of tritiated water in the blood during the first 5 min after its oral administration to 13 rats that had received a single oral dose of 1·5 g EAE/kg 0–72 hr previously. Water intake, urine output and haematocrit were determined in 32 rats at 0–96 hr after an oral dose of 2 g EAE/kg. Sodium and potassium levels in plasma and sodium levels in urine were determined by flame photometry in 12 rats at 0–72 hr after an oral dose of 2 g EAE/kg. A group of ten rats received fluid replacement by intraperitoneal injections of 3 ml Tyrode's fluid on days 2, 3 and 4 after administration of an oral dose of 2 g EAE/kg and the effect on mortality was studied for 15 days thereafter.

Feeding studies

30-Day study. Groups of ten male rats (body weight approximately 290 g) were given neutralized EAE in their drinking water, provided *ad lib*, at levels of 0, 250, 500, 1000, 2000 or 4000 ppm for 30 days. Body weight and water intake were recorded 3 times/wk. Blood was taken from all animals surviving the 30-day treatment for determinations of haemoglobin concentration, haematocrit, red and white cell counts, urea-nitrogen content and glutamic–oxalacetic transaminase activity. The urea-nitrogen concentration was determined by the method of Gentzkow (1942) and transaminase activity by the method of Steinberg, Baldwin & Ostrow (1956). At autopsy, the brain, liver, kidneys and spleen of all animals were weighed and gross and histological examinations were carried out.

90-Day study. Groups of 20 male rats (body weight approximately 170 g) were given drinking water *ad lib.* containing 0, 25, 50, 100, 200 or 400 ppm EAE for 90 days. The scope of the investigation was similar to that described for the 30-day study except that the blood examination was confined to determinations of the haematocrit and glutamic–oxalacetic transaminase.

RESULTS

Acute toxicity

The acute oral LD_{50} of neutralized EAE was 1·61 g/kg (95% fiducial limits: 1·50–1·72 g/kg) in male rats. EAE was more toxic to female rats, the oral LD_{50} being 1·02 (0·90–1·16) g/kg. The intraperitoneal LD_{50} in male rats was 1·18 (1·03–1·34) g/kg. Following a lethal oral dose of EAE, deaths did not occur until 3 days after administration. The stomachs were greatly distended with fluid. No significant histopathological changes were evident in the stomach, liver, spleen and duodenum. There were occasional proteinaceous casts in the kidney tubules 48 hr after oral administration of EAE. After an intraperitoneal dose of EAE most deaths occurred within 24 hr but no significant pathological changes were noted.

After a lethal (2 g/kg) oral dose of neutralized EAE, the gastro-intestinal tract became filled with fluid, but no histopathological change was evident. Gastric secretion was significantly increased by an oral dose of 1·5 g EAE/kg but not by its diethyl analogue (Table 1). Acid secretion was stimulated to a greater extent than fluid secretion.

There was apparently no compensatory increase in fluid absorption, as indicated by the progressively reduced rate of absorption of tritiated water after EAE treatment. The uptake

Table 1. *Gastric secretion 3 days after an oral (1·5 g/kg) dose of EAE or its 2-N-diethyl analogue*

Interval after ligation (min)	Control	EAE	2-*N*-diethylamino-ethanol
	Acid secretion (μ-equiv. HCl/rat)		
10–40	108 ± 11	527 ± 28**	223 ± 14
40–70	112 ± 24	515 ± 39**	126 ± 17
70–100	85 ± 28	502 ± 30**	94 ± 6
	Fluid secretion (ml/rat)		
10–40	2·34 ± 0·55	4·88 ± 0·81*	2·90 ± 0·13
40–70	1·51 ± 0·22	4·38 ± 0·55**	2·22 ± 0·34
70–100	1·45 ± 0·36	4·00 ± 0·39**	1·75 ± 0·17

Values marked with asterisks are significantly greater than those of controls: *$P < 0·05$; **$P < 0·01$.

rates at 24, 48 and 72 hr after an oral dose of 1·5 g EAE/kg were 43·3 ± 11·0, 16·0 ± 6·8 and 11·3 ± 24·4 cpm/min^2 compared with a control rate of 68·8 ± 19·5 cpm/min^2.

As the stomachs of EAE-treated rats filled with secretory fluids, their water intake decreased (Table 2), the haematocrit increased markedly, and the urine output dropped almost to zero. Death was probably due to dehydration resulting from the pronounced fluid shift into the gastro-intestinal tract.

Fluid replacement by intraperitoneal injections of 3 ml Tyrode's fluid on days 2, 3 and 4 after dosing with EAE reduced the lethal effect of EAE (Table 3).

Table 2. *Water intake, urine output and haematocrit value in rats 0–96 hr after an oral dose of 2 g EAE/kg*

Time after dosing (hr)	Water intake (ml)	Urine output (ml)	Haematocrit (%)
0	38·75 ± 2·20	18·50 ± 1·21	46·8 ± 0·4
24	21·06 ± 1·38**	19·19 ± 1·13	46·9 ± 0·6
48	17·96 ± 2·02**	15·36 ± 1·42*	52·3 ± 1·2**
72	3·56 ± 0·74**	4·12 ± 0·41**	61·0 ± 0·4**
96	0·77 ± 0·34**	4·8	62·1

Values marked with asterisks differ significantly from those of controls: *$P < 0·05$; **$P < 0·01$.

Table 3. *Cumulative mortality of rats given fluid replacement with Tyrode's solution on days 2–4 after an oral dose of 2 g EAE/kg*

Group	Cumulative mortality (%) at day						
	3	4	5	6	10	13	15
Without fluid replacement	90	100	100	100	100	100	100
With fluid replacement	40	60	70	70	70	80	80

In spite of the pronounced fluid shifts, plasma sodium and potassium concentrations showed only minor changes (Table 4).

Table 4. *Cationic concentrations in plasma and urine at 0–72 hr after an oral dose of 2 g EAE/kg in rats*

Time after dosage (hr)	Plasma Na^+ (m-equiv./l)	Plasma K^+ (m-equiv./l)	Urine Na^+ (m-equiv./l)
0	144 ± 2	5·54 ± 0·27	90 ± 12
24	143·5 ± 2·5	5·82 ± 0·15	95·2 ± 14·8
48	138·2 ± 2·7	5·71 ± 0·28	108·8 ± 14·3
72	141·7 ± 6·1	6·20 ± 0·54	78·2 ± 8·4

30-Day feeding study

All animals given 4000 or 2000 ppm EAE in the drinking water died within 15 and 17 days respectively. Some deaths also occurred at the 500 and 1000 ppm levels. The LC_{50} over 30 days was 1412 ± 710 ppm. Animals on 250–1000 ppm EAE grew as well as the controls for the first 10 days but thereafter growth was retarded at 250 and 500 ppm and weight loss occurred at 1000 ppm (Fig. 1). Regular determination of the water intake

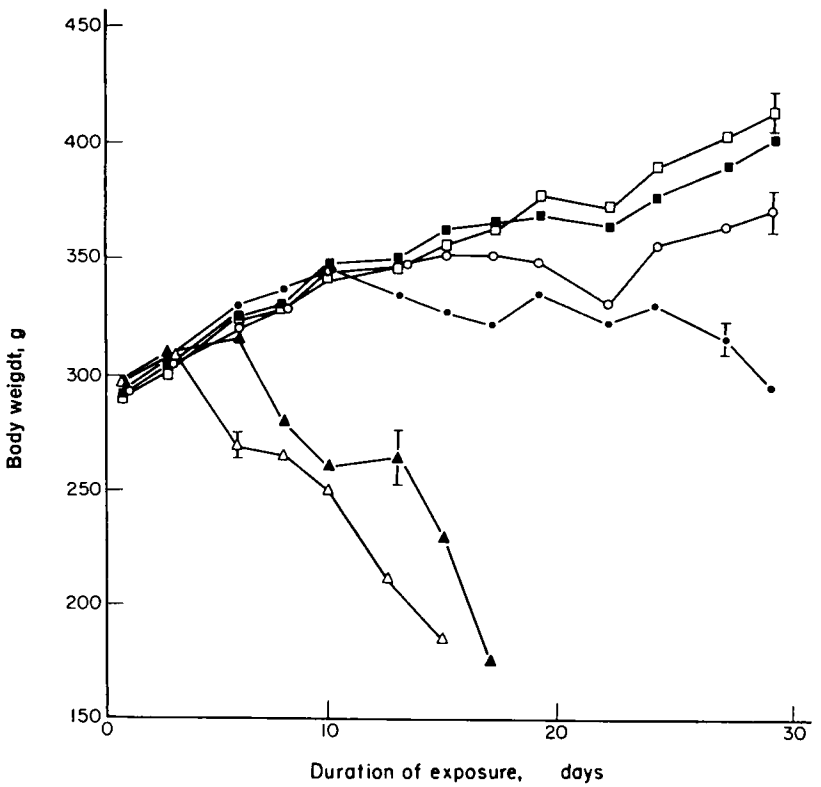

FIG. 1. Body weights of male rats given EAE in the drinking water at levels of 0 (□), 250 (■), 500 (○), 1000 (●), 2000 (▲) or 4000 (△) ppm for up to 30 days.

enabled calculation of the average cumulative dose of EAE until death and the equivalent dose for the survivors (Table 5). The data indicate that EAE has a marked cumulative effect when administered in the drinking water. However, except for pronounced diarrhoea at the higher dose levels, the effect of EAE in this 30-day feeding study differs from that produced by a single large dose of EAE.

Table 5. *Cumulative doses in rats receiving 250–4000 ppm EAE in the drinking water for 30 days*

EAE level (ppm)	Total dose to death (mg)	Total dose in survivors (mg)
4000	864	—
2000	750	—
1000	720	1269
500	593	716
250	—	355

At the end of the 30-day period of feeding, the stomachs were not distended with fluid and could not be differentiated from their controls by gross examination. There was no significant indication of haemoconcentration at any dose level. Organ weight to body weight ratios showed significant increases for kidneys, spleen and brain in the 500 and 1000 ppm group (Table 6). These changes can be attributed in part to losses in body weight during the experiment. The white cell count showed a dose-related increase. Serum glutamic–oxalacetic

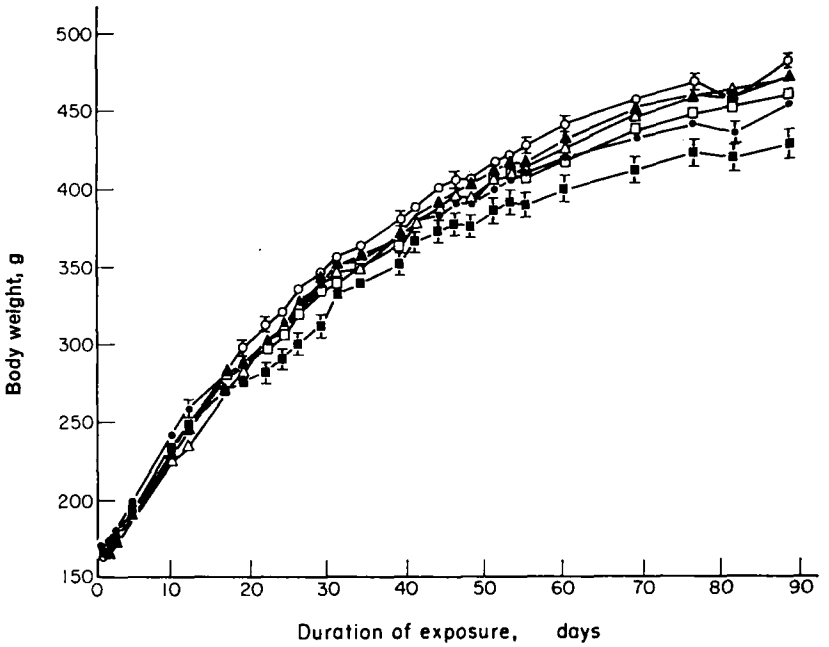

FIG. 2. Body weights of male rats given EAE in the drinking water at levels of 0 (○), 25 (▲), 50 (△), 100 (□), 200 (●) or 400 (■) ppm for up to 90 days.

Table 6. *Terminal findings in 30-day EAE feeding study in rats*

Level of EAE (ppm)	Organ weight/body weight = 1000				Haematocrit (%)	Hb (g/100 ml)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	BUN (mg/100 ml)	SGOT (OD units)
	Brain	Liver	Kidneys	Spleen						
0	4·12 ± 0·21	42·2 ± 0·9	7·01 ± 0·12	2·11 ± 0·04	46·5 ± 0·8	16·6 ± 0·4	8·15 ± 0·26	10·6 ± 0·8	16·5 ± 0·5	222 ± 20
250	4·07 ± 0·25	43·1 ± 0·8	6·59 ± 0·15*	2·15 ± 0·07	43·0 ± 0·6**	15·3 ± 0·2**	8·53 ± 0·24	12·7 ± 0·4*	19·6 ± 1·5*	214 ± 11
500	4·63 ± 0·16*	42·7 ± 0·9	8·17 ± 0·37**	2·31 ± 0·08*	46·8 ± 0·6	17·3 ± 0·3	8·98 ± 0·39*	13·7 ± 1·2*	16·6 ± 0·6	310 ± 31*
1000	5·97 ± 0·14**	42·9 ± 1·1	10·04 ± 0·30**	2·84 ± 0·30*	47·6 ± 1·0	17·5 ± 0·5	9·06 ± 0·26*	17·8 ± 2·0**	34·0 ± 9·4*	352 ± 53*

Hb = Haemoglobin RBC = Red blood cells WBC = White blood cells
BUN = Blood urea-nitrogen SGOT = Serum glutamic-oxalacetic transaminase

Values are means for all survivors and those marked with asterisks differ significantly (Student's *t* test) from those of controls: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$.

Table 7. *Terminal findings† in 90-day EAE feeding study in rats*

Level of EAE (ppm)	Organ weight/body weight × 1000				Terminal body weight (g)	Haematocrit (%)	SGOT (OD units)
	Brain	Liver	Kidneys	Spleen			
0	3·55 ± 0·08	35·7 ± 0·5	6·86 ± 0·11	1·88 ± 0·04	500 ± 6	50·9 ± 0·5	148 ± 10
25	3·74 ± 0·07*	35·8 ± 0·5	6·46 ± 0·12**	1·75 ± 0·04*	489 ± 7	48·5 ± 0·4**	160 ± 9
50	3·60 ± 0·09	34·3 ± 0·6*	6·75 ± 0·12	1·86 ± 0·04	480 ± 8*	48·2 ± 0·6**	200 ± 14**
100	3·60 ± 0·12	34·1 ± 0·6*	6·27 ± 0·07**	1·88 ± 0·04	475 ± 7**	47·3 ± 0·5**	210 ± 19**
200	3·74 ± 0·07*	36·9 ± 0·6	6·43 ± 0·08**	1·89 ± 0·04	464 ± 7**	44·7 ± 0·8**	192 ± 10**
400	4·02 ± 0·19*	40·7 ± 1·9**	6·88 ± 0·29	2·85 ± 0·18**	434 ± 13**	50·2 ± 1·1	406 ± 53**

SGOT = Serum glutamic-oxalacetic transaminase

Values marked with asterisks differ significantly (Student's *t* test) from those of controls: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$.

† Values are means for groups of 20 rats, except those relating to 100 and 400 ppm EAE, which are means for groups of 19 rats.

transaminase levels were increased at the 500 and 1000 ppm levels and blood urea-nitrogen levels were increased at 1000 ppm (Table 6).

Histopathological changes were most evident in the kidney. The damage to this organ was dose related, ranging from minimal cloudy swelling of the tubular epithelium at 250 ppm to extensive cloudy swelling with degenerative changes in the tubular epithelium at 1000 ppm. At 1000 ppm the villi in the duodenum were conspicuously shortened. Tissue sections of brain, stomach, spleen, liver, lungs and pancreas did not differ significantly from those of controls.

In the 90-day feeding study, using lower levels of EAE, growth retardation became more apparent with increasing dose level (Fig. 2) and was marked in the groups given the three highest dose levels. There were two deaths during the experiment, one at the 400 ppm level and another at the 100 ppm level. At autopsy, there were no gross changes in any of the groups, but the relative weights of the liver, brain and spleen were increased at the highest dose level (Table 7). There were no significant histopathological changes in the small intestine, spleen or pancreas. At the 400 ppm level, the kidney tubules contained occasional proteinaceous casts. Some of the livers of rats at the 400 ppm level showed binucleated cells and variability in nuclear size. At high dose levels the fat stains of the liver sections were uniformly negative. Serum glutamic–oxalacetic transaminase levels were elevated at and above 50 ppm.

Calculation of the total intake of EAE during the 90-day feeding study on the basis of the observed water consumption showed that the rats at the 400 ppm level ingested a total of 1·69 g EAE, with other rats consuming proportionately less.

DISCUSSION

Single lethal oral doses of EAE produce profound alterations in fluid balance. Increased gastric secretion results in generalized dehydration and death after 3–4 days. This effect is probably not due to osmotic effects, because equivalent amounts of 2-*N*-diethylaminoethanol do not produce the same result. Neither are there any histopathological lesions of the gastro-intestinal tract. Therefore it appears that EAE is able either to affect fluid transport in the gastro-intestinal tract directly, or to influence the nervous or hormonal control of gastric secretion. Bell, Davis & Strength (1964) have shown that EAE can be incorporated into phospholipids and methylated to the corresponding phosphatidylcholine analogue. Since phospholipids appear to be involved in the transport of substances across cell membranes, it is possible that EAE interferes with the normal functioning of phospholipids and affects the transport of fluids. The long duration of the secretory response after EAE argues against its direct effects on humoral mechanisms analogous to those of ethanol (Hirschowitz, Pollard, Hartwell & London, 1956).

The effects of 90-day administration of EAE in the drinking water are more diffuse. At the higher concentrations of EAE there are indications of alterations in fluid balance of a relatively minor nature. The main effects are growth retardation and degenerative changes, but the changes in the liver and kidney do not appear to be sufficiently severe to account for the observed mortalities.

The highly cumulative nature of EAE toxicity is compatible with substitution of EAE in the normal metabolic pathways of 2-aminoethanol or 2-methylaminoethanol and subsequent functional and structural changes. The observed cumulative effects of oral EAE exposure demand caution in its long-term use.

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Toxicité aiguë et à court terme du 2-*N*-éthylamino-éthanol administré par voie orale à des rats

Résumé—Des doses orales aiguës de 2-*N*-éthylamino-éthanol (EAE) neutralisé ont fait augmenter considérablement la sécrétion gastrique chez les rats, provoquant en 3–4 jours la déshydratation et la mort. L'action de l'EAE sur la sécrétion gastrique n'a pas semblé être imputable à des effets osmotiques ou à des altérations histopathologiques du tractus gastro-intestinal.

L'administration à court terme d'EAE neutralisé ajouté à l'eau de boisson a fourni la preuve d'une toxicité cumulative. La CL_{50} à 30 jours était de 1412 $\pm$ 710 ppm. Les effets toxiques majeurs de l'EAE administré pendant une période de 90 jours à des concentrations plus faibles ont été un ralentissement de la croissance et des altérations dégénératives du foie et des reins. L'administration pendant 90 jours de 25 ppm d'EAE dans l'eau de boisson n'a provoqué que des modifications mineures; ce taux est considéré comme très proche du seuil d'indifférence.

Akute und kurzzeitige orale Toxizität von 2-*N*-Äthylaminoäthanol in Ratten

Zusammenfassung—Akute orale Dosen von neutralisiertem 2-*N*-Äthylaminoäthanol (ÄAÄ) riefen in Ratten eine starke Steigerung der Magensekretion hervor, die zur Dehydratation und zum Tode nach 3–4 Tagen führte. Der Einfluss von ÄAÄ auf die Magensekretion schien nicht auf osmotische Vorgänge oder auf histopathologische Veränderungen im Verdauungstrakt zurückzuführen zu sein.

Kurzzeitige Verabreichung von neutralisiertem ÄAÄ im Trinkwasser lieferte Beweise für deutliche kumulative Toxizität. Die LC_{50} für 30 Tage betrug 1412$\pm$710 ppm. Bei geringeren Konzentrationen, die 90 Tage lang angewendet wurden, waren die toxischen Hauptwirkungen von ÄAÄ Wachstumsverzögerung und degenerative Veränderungen in der Leber und den Nieren. Eine Konzentration von 25 ppm ÄAÄ im Trinkwasser für 90 Tage rief kleinere Veränderungen hervor und wurde als nahe der wirkungsfreien Konzentration angesehen.