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# Acute Toxicity for Rats and Mice of 2-Ethyl Hexanol and 2-Ethyl Hexyl Phthalate.*

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Since 1869 the phenomenon of increasing toxicity of alcohols with increasing molecular weight has been referred to as Richardson's Law.[1] Considerable data in detail on many alcohols have appeared in the last twenty years, and, in general, the results confirm Richardson's observations.[2-12] Secondary alcohols have been shown to be less toxic than the corresponding primary alcohols. However, no investigation has been sufficiently comprehensive to include simultaneous measurements on straight and branched chain, primary and higher alcohols.

No published data are available on the toxicity of 2-ethyl hexanol. However, it appears that this branched chain octanol has, in rats, about the same order of toxicity as ethyl alcohol in cats (intraperitoneal administration). Ethyl hexanol given into the stomach seems to be only one-sixth as toxic in rats as ethyl alcohol, given the same way, is in rabbits.

*Method.* The acute toxicity of ethyl hexanol was measured on groups of male and female adult albino rats. Two routes of administration were used (a) intraperitoneal injection, and (b) stomach tube administration. The 2-ethyl hexanol was taken from a 3 kg sample of Eastman Kodak Company, practical grade.

*Intraperitoneal Injection in Rats.* In a preliminary experiment groups of 5 rats each were injected with various doses of ethyl hexanol intraperitoneally. The lethal dose for the average rat lay between 0.1 and 0.2 cc. On the basis of this information, groups of about 15 rats each were given doses ranging from 0.10 to 0.24 cc of ethyl hexanol (Table I, A). The percentage mortality-dosage curve was a typical s-shaped curve without undue scatter. The LD 50, calculated by the method of Bliss, was 0.078 cc per 100 g rat or 0.65 g per kg body weight. This represents a moderate toxicity.

Rats given ethyl hexanol intraperitoneally promptly developed an irregular gait and exhibited dragging of the hind legs. In the next few minutes after high doses, the breathing became gasping in character, although cyanosis was not noted. Insensibility rapidly followed and even on the lower doses (0.02 cc per rat) all the rats were sound asleep in 10 minutes. It appeared that ethyl hexanol has marked anesthetic properties.

*Stomach Tube Administration to Rats.* In a preliminary experiment on groups of 5 rats each, it was found that the lethal dose lay between 0.6 and 1.0 cc. Consequently large groups of rats (Table I, B) were given doses as shown. The percentage mortality-dosage curve is a smooth s-shaped curve and the LD 50 calculated according to the method of

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TABLE I.
Acute Toxicity of 2-Ethyl Hexanol in Rats and Mice.

| No. rats | Avg body wt, g | Dose, cc | No. dead | % mortality |
|---|---|---|---|---|
| | A. Intraperitoneal Injection (101 rats). | | | |
| 5 | 177 | .02 | 0 | 0 |
| 5 | 193 | .05 | 0 | 0 |
| 15 | 269 | .10 | 2 | 13 |
| 15 | 234 | .14 | 3 | 20 |
| 15 | 197 | .16 | 6 | 40 |
| 15 | 208 | .20 | 13 | 87 |
| 16 | 206 | .24 | 14 | 88 |
| 10 | 345 | .40 | 7 | 70 |
| 5 | 217 | .40 | 12 | 100 |
| LD 50 (probit kill — 5.0) = 0.078 cc per 100 g rat. | | | | |
| | B. Stomach Tube Administration (85 rats). | | | |
| 5 | 183 | .05 | 0 | 0 |
| 5 | 207 | .20 | 0 | 0 |
| 15 | 230 | .60 | 3 | 20 |
| 15 | 189 | .80 | 9 | 60 |
| 15 | 184 | 1.0 | 11 | 73 |
| 15 | 179 | 1.6 | 14 | 93 |
| 15 | 294 | 2.0 | 15 | 100 |
| LD 50 (probit kill — 5.0) = 0.39 cc per 100 g rat. | | | | |
| | C. Intraperitoneal Injection (102 mice). | | | |
| 5 | 22 | .005 | 0 | 0 |
| 5 | 24 | .010 | 0 | 0 |
| 15 | 23 | .015 | 2 | 13 |
| 15 | 23 | .020 | 9 | 60 |
| 17 | 23 | .025 | 16 | 94 |
| 15 | 23 | .030 | 15 | 100 |
| 30 | 23 | .05+ | 30 | 100 |

Bliss is 0.39 cc per 100 g rat or 3.2 g per kg body weight. Thus for the average rat, about 5 times as much ethyl hexanol must be given orally to produce death than is required intraperitoneally.

*Intraperitoneal Injection in Mice.* In Table I C are given the results from the administration of various doses of 2-ethyl hexanol intraperitoneally to mice. The ethyl hexanol was administered in a 1:10 dilution in mazola. This vehicle caused no apparent change in the symptoms produced by the ethyl hexanol; the mice promptly went to sleep as did the rats which received undiluted ethyl hexanol. The dose to kill the average mouse is of the order of 0.018 cc or 0.78 g per kg body weight. The lethal dose for mice is of the same magnitude as that for rats following intraperitoneal injection.

*Acute Toxicity of 2-Ethyl Hexyl Phthalate.* The toxicity of phthalates has been previously shown to decrease with increasing molecular weight of the alkyl component.[13] That the toxicity is also a function of the nature of the alkyl moiety is indicated from the current finding that 2-ethyl hexyl phthalate is much less toxic than di-octanol-2 phthalate.

*Stomach Tube Administration to Rats.* In Table II A are given the data on a total of 50 rats given various doses of 2-ethyl hexyl phthalate. No fatalities were obtained. "Diarrhea" was frequently produced by the largest doses. However, the drug caused a sweeping out of the gastro-intestinal tract and not the production of liquid feces. Twenty-four hours later the gastro-intestinal tracts contained formed feces, and the feces pellets which had accumulated were firm not soft. The maximum dose of 34 g per kg body weight corresponds to a dose of over 2 liters for a 70-kg man, and indicates the extremely low toxicity of ethyl hexyl phthalate in rats.

On autopsy the organs in the peritoneal cavity were normal-appearing. The only peculiarity was the engorgement of the stomachs which seemed perhaps twice as large as filled

[13] Hodge, H. C., Goldstein, M. R., and Wrightington, H., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 471.

TABLE II.
Acute Toxicity of 2-Ethyl Hexyl Phthalate in Rats and Mice.

| No. rats | Avg body wt | Dose g/kilo | Effects |
|---|---|---|---|
| | A. Stomach Tube Administration (50 rats). | | |
| 15 | 136 | 34.5 | Diarrhea |
| 15 | 137 | 13.7 | " |
| 5 | 200 | 4.5 | " |
| 5 | 214 | 2.8 | None |
| 5 | 205 | 2.0 | " |
| 5 | 189 | 1.1 | " |
| | B. Intraperitoneal Injection (50 rats). | | |
| 15 | 198 | 23.8 | Diarrhea |
| 15 | 196 | 19.2 | Some diarrhea |
| 5 | 155 | 18.1 | ? |
| 5 | 149 | 12.7 | None |
| 5 | 159 | 5.7 | " |
| 5 | 159 | 3.1 | " |
| | C. Intraperitoneal Injection (65 mice). | | |
| No. mice | Avg body wt | Dose g/kilo | No. dead | % mortality |
| 25 | 19 | 50 | 3 | 12 |
| 20 | 22 | 86 | 3 | 15 |
| 20 | 22 | 128 | 1 | 5 |

stomachs ordinarily appear. Perhaps the cause lay in a temporary starvation following the administration of ethyl hexyl phthalate which was then followed by a period of gorging. However, some slowing of the pyloric emptying may have resulted.

*Intraperitoneal Injection in Rats.* In Table II B data are given on the intraperitoneal injection of ethyl hexyl phthalate to a total of 50 rats. No deaths were observed although one group of 15 rats received about 24 g of ethyl hexyl phthalate per kg body weight. The only effects noted were evidences of diarrhea.

On autopsy the peritoneal cavities contained a milky watery emulsion and the livers seemed to be unusually large and firm. Consequently a group of 5 rats were killed on the third day after receiving ethyl hexyl phthalate, 5 additional rats were killed two days later, and 6 rats were killed 10 days later. The average liver weights were 8.8, 7.8, and 7.3 g, respectively. Since the last figure was nearer the average liver weight in normal rats, it may be assumed that intraperitoneal injection of ethyl hexyl phthalate produced some sort of toxic reaction in the livers, which effect was subsiding during the 10-day period. Liver sections were taken from each rat and stained with hematoxylin and eosin. The following report was submitted by J. R. Carter of the Department of Pathology:

"All sections show definite cytoplasmic changes consisting of increased swelling of cells with marked granularity and moderate dissolution and degeneration. All areas have a decided foamy appearance—both portal and central areas being equally involved. In many areas, the sinusoids are obliterated by the swollen cells. No areas of necroses are seen and nuclear changes, if any, are minimal. The bile canliculi are normal.

"Impression: No specific diagnosis can be attached to these findings except, perhaps that of cloudy swelling. The process is diffuse and the vacuolated areas producing the foamy appearance is due to some substance which will not stain with hematoxylin and eosin."

*Intraperitoneal Injection in Mice.* Groups of 20 to 25 mice each were given various doses of ethyl hexyl phthalate by intraperitoneal injection (Table II, C). Of the 25 mice receiving 1 cc, 3 died; of the 20 mice receiving 2 cc, 3 died; and of the 20 mice receiving 3 cc, one died. On the largest dosage the mice received 128 g per kg body weight, which would correspond to more than 9 liters for a 70-kg man. The fact that the mortality does not increase with the dosage emphasizes the low toxicity of ethyl hexyl phthalate in mice.

*Summary.* 1. The acute toxicity of 2-ethyl hexanol following intraperitoneal injection in 101 rats has been measured. The LD 50 was

found to be of the order of 0.08 cc per 100 g rat. From the administration of 2-ethyl hexanol by stomach tube to 85 rats, the LD 50 has been shown to be of the order of 0.39 cc per 100 g rat.

2. A marked anesthetic quality of 2-ethyl hexanol was observed.

3. 2-ethyl hexyl phthalate given by stomach tube to 50 rats in doses of 1 to 34 g per kg body weight produced no deaths. 2-ethyl hexyl phthalate has a very low order of toxicity in rats.

4. 2-ethyl hexyl phthalate given intraperitoneally in doses of 1, 2, and 3 cc respectively, to 65 mice resulted in the death of 7. However, only one mouse in a group of 20 was killed by the largest dose (128 g per kg body weight). 2-ethyl hexyl phthalate has a very low order of toxicity in mice.

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## Probable Mechanism by Which Somatic Changes in Certain Emotional States Are Mediated.*

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Somatic changes commonly occur as the result of emotional reactions such as fear, tension and anxiety. Among the somatic changes are sweating, tachycardia, elevation of blood pressure, leucocytosis, increased intestinal peristalsis and alterations in metabolism of glucose and in skin temperature. Results of investigations on certain of these changes were reported by Diethelm[1] on glucose metabolism, Mittelmann and Wolff[2] on skin temperature, and Milhorat, Small and Diethelm[3] on leucocytosis.

In the present investigations an attempt was made to understand the mechanism or mechanisms by which these somatic changes are mediated. Many of the changes resemble those induced by the administration of adrenalergic and cholinergic drugs. In fact, Cannon and de la Paz[4] observed that blood withdrawn from the suprarenal veins of cats during periods of fear had adrenalergic effects on a surviving strip of rabbit intestine, whereas blood drawn while the animals were quiet had no such effect. The method used in the present studies represents a modification of the procedure of Cannon and is described below. The method of determination of adrenalin in the blood by colorimetric means,[5] was found unsatisfactory for our purposes.

*Methods and Materials.* Healthy adult rabbits were killed by a blow on the head. The intestines were removed immediately and with careful handling were washed in saline. A loop of intestine was suspended in Ringer-Tyrode's solution, attached to a recording lever and the contractions were recorded on a kymograph. The solution was gently agitated throughout the experiment by means of a stream of air bubbles. The bottle containing the muscle strip and Ringer-Tyrode's solution was suspended in a water bath at 37°C.

The blood was drawn from the patient's cubital vein, mixed immediately with heparin in a small Erlenmyer flask and within a period

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