E. C., *Trans. N. Y. Acad. Sci.*, 1962, Ser. II, v24, 504.

2. Bloom, W. L., Watson, D. W., Cromartie, W. J., Freed, M., *J. Inf. Dis.*, 1947, v80, 41.

3. Dubos, R. J., Hirsch, J. G., *J. Exp. Med.*, 1954, v99, 55.

4. Crampton, C. F., Moore, S., Stein, W. H., *J. Biol. Chem.*, 1955, v215, 787.

5. Li, C. P., Prescott, B., Chi, L. L., Martino, E. C., *Fed. Proc.*, 1963, v22, Part I, No. 2, 208.

6. Limasset, P., Truffaut, L., *Compt. rend Acad. Sci.*, 1963, v256, 2258.

7. Mattern, C. F. T., Chi, L. L. *Virology*, 1962, v18, 257.

8. Szent-Györgyi, A., Hegyeli, A., McLaughlin, J. A., *Proc. Nat. Acad. Sci.*, 1962, v48, 1439.

9. Hegyeli, A., McLaughlin, J. A., Szent-Györgyi, A., *Fed. Proc.*, 1963, v22, Part I, No. 2, 570.

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Comparison of Acute Toxicity of Anticholinesterase Insecticides to Weanling and Adult Male Rats.* (28716)

JULES BRODEUR† AND KENNETH P. DUBOIS

Department of Pharmacology, University of Chicago, Chicago, Ill.

The acute toxicity of insecticides is usually studied in adult animals. Little information is therefore available on the susceptibility of young animals to these compounds. Previous studies(1,2) have demonstrated that young animals are much more susceptible than adults to some organophosphates. Thus the acute toxicity of O-ethyl O-(4-nitrophenyl) benzene thionophosphonate (EPN)(1) and O,O-diethyl O-(4-methylthio-m-tolyl) phosphorothioate (DMP, Bayer 29492)(2) was found to be much higher in 23-day-old male rats than in the adult rats of the same sex. The acute toxicity of the various insecticides now approved for use on food crops has not been studied systematically in the young rat. The present study was undertaken to obtain a comparison of the acute toxicity of several commonly used insecticides of the anticholinesterase series to weanling and adult male rats under similar experimental conditions.

Materials and methods. Weanling (23-day-old, 50 to 60 g) and adult (200 to 300 g) male Holtzman rats were used for these experiments. The toxicity of the following insecticides was measured: O,O-diethyl O-(4-nitrophenyl) phosphorothioate (parathion), O,O-dimethyl O-(4-nitrophenyl) phosphorothioate (methyl parathion), O-ethyl O-(4-nitrophenyl) benzene thionophosphonate (EPN), mixture of O,O-diethyl O-(2-ethylthio)ethyl phosphorothioate and O,O-diethyl S-2-(ethylthio)ethyl phosphorothioate (Systox), O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate (Co-Ral), O,O-diethyl S-2-(ethylthio)ethyl phosphorodithioate (Di-Syston), O,O-diethyl S-(p-chlorophenylthio)-methyl phosphorodithioate (Trithion), O,O,O,O-tetraethyl S,S′-methylene *bis*-phosphorodithioate (Ethion), O,O-dimethyl S-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate (Guthion), O,O-dimethyl S-[1,2-*bis*-(ethoxycarbonyl)ethyl] phosphorodithioate (Malathion), 2,3-p-dioxanedithiol-S,S-*bis*-(O,O-diethyl phosphorodithioate) (Delnav), O,O-dimethyl 1-methyl-2-carbomethoxyvinyl phosphate (Phosdrin), tributyl phosphorotrithioite (Folex), dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate (Dipterex), octamethyl pyrophosphoramide (OMPA), and 1-naphthyl-N-methylcarbamate (Sevin). With the exceptions indicated below, the compounds were dissolved in ethanol (20%) and propylene glycol (80%) and the amount of toxicant in solution was adjusted so that an amount equivalent to 0.2% of the body weight was given intraperitoneally to weanlings and an amount equivalent to 0.1% was administered by the same route to the adults. Undiluted Malathion was given to adults. Dipterex was dissolved in distilled water. Co-Ral was used

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† Medical Research Fellow, Medical Research Council, Canada.

TABLE I. Comparison of Acute Toxicity of Anticholinesterase Insecticides to Weanling and Adult Male Rats.

Insecticide	Weanlings			Adults		
	No. of rats	LD_{50} (mg/kg)	19/20 confidence limits (mg/kg)	No. of rats	LD_{50} (mg/kg)	19/20 confidence limits (mg/kg)
Parathion	27	1.5	(1.2 – 1.9)	23	3.6	(3.1 – 4.2)
Methyl parathion	58	3.5	(2.8 – 4.4)	24	5.8	(5.0 – 6.7)
EPN	61	8.0	(7.0 – 9.1)	34	33	(28 – 40)
Systox	20	2.5	(2.1 – 2.9)	16	3.8	(3.1 – 4.7)
Co-Ral	30	145	(99 –213)	20	180	(161 – 202)
Di-Syston	25	5.4	(5.0 – 5.8)	30	9.4	(8.5 – 10.3)
Trithion	43	11	(9.4 – 11.8)	18	40	(31 – 52)
Ethion	35	100	(92 –109)	22	128	(110 – 149)
Guthion	24	3.4	(3.1 – 3.7)	20	4.9	(4.1 – 5.8)
Malathion	20	340	(236 –490)	18	750	(544 –1035)
Delnav	43	49	(31 – 78)	30	94	(70 – 126)
Phosdrin	35	.89	(.85– .93)	18	1.35	(1.23– 1.48)
Folex	24	76	(63 – 91)	28	150	(105 – 215)
Dipterex	30	190	(155 –234)	21	250	(200 – 313)
OMPA	25	49	(42 – 58)	25	10	(8.1 – 12.3)
Sevin	26	48	(41 – 56)	16	64	(52 – 79)

as an aqueous suspension in 0.2% carboxymethylcellulose. Folex was given as a suspension in ethanol and propylene glycol. A 14-day observation period was used. Death or apparent complete recovery occurred with all of the compounds within the first 7 days after administration. The LD_{50} of each compound with 19/20 confidence limits was calculated by the method of Litchfield and Wilcoxon(3).

Results. After administration of each of the insecticides, symptoms typical of those resulting from cholinesterase inhibition were observed with all of the compounds. The data in Table I show that the toxicity of parathion, methyl parathion, Systox and EPN is significantly greater in weanling male rats than in adult males. The greatest difference among the phosphorothioates was seen with EPN to which weanlings were about 5 times more susceptible than adult males. The phosphorodithioates included in this study were all more toxic to weanlings than to adults. Thus, Di-Syston, Malathion and Delnav were about twice as toxic to weanlings as they were to adults. A smaller age difference was noted with Ethion and Guthion. Weanling rats were approximately 4 times more susceptible to the acute toxicity of Trithion than adult males.

Only a slight increase in susceptibility of weanling rats as compared with adults was noted with Phosdrin, Dipterex, and Sevin. Folex was about twice as toxic to weanlings as it was to adults. OMPA differed from all of the other compounds in that adult males were about 5 times more susceptible than weanlings.

Discussion. This investigation was undertaken to compare the susceptibility of young and adult male rats to a number of anticholinesterase insecticides which are permitted for use on various food crops intended for human consumption. Our results indicate that most of the compounds are more toxic to weanling male rats than to adults. Since all of the young animals used for this study were 23 days of age, further studies are indicated to ascertain whether the susceptibility is further increased at an age of less than 23 days. The permitted levels (tolerance levels) of pesticides in food have been established on the basis of feeding studies initiated with weanling rats. The possible increased susceptibility at earlier ages has thus not been studied as part of the experimental work preceding establishment of tolerance levels for pesticides.

The mechanisms responsible for age differences in susceptibility of male rats to cholinergic organic phosphates are not clear. Most of the compounds which exhibit age differences in toxicity must undergo metabolic conversion to become anticholinesterase agents. However, the rate of conversion to an active metabolite is slower in the livers

of weanlings than in adults(1). Differences in rate of activation, therefore, cannot account for the results obtained here except in the case of OMPA where the susceptibility of adult and weanlings may be explainable on the basis of incomplete development of the microsome oxidase which catalyzes the activation of this compound.

Previous studies(2) have provided substantial evidence that some phosphorothioates are detoxified by the catalytic action of microsome enzymes and that the toxicity of some phosphorothioates and dithioates is governed to various degrees by these metabolic reactions. It has been well established that the microsome enzymes that catalyze drug metabolism develop after birth of some species of experimental animals(4). It appears that rate and extent of detoxification of certain of the anticholinesterase insecticides more closely parallels the liver microsome enzyme levels than does the activation reaction which the compound must also undergo to exhibit anticholinesterase activity. It appears that activation of the compounds requires only a small fraction of the total amount of microsome oxidase activity in the adult liver. Thus rate of detoxification would tend to have a marked influence on toxicity of the compounds.

Summary. A comparison was made of the acute toxicity of a number of anticholinesterase insecticides in weanling and adult male rats. The results indicate that weanlings are about twice as susceptible as adults to parathion, methyl parathion, Systox, Di-Syston, Guthion, Malathion, Delnav, and Folex. A smaller increase in susceptibility of weanlings was noted with Ethion, Phosdrin, Dipterex and Sevin. Weanlings were about 5 times more susceptible to EPN and about 4 times more susceptible to Trithion than adult male rats. In the case of OMPA adults were about 5 times more susceptible than weanlings. The age differences in susceptibility are probably due to incomplete development of enzymes which catalyze the metabolism of anticholinesterase insecticides in the livers of young animals.

1. Murphy, S. D., DuBois, K. P., *J. Pharm. Exp. Therap.*, 1958, v124, 194.

2. DuBois, K. P., Puchala, E., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 908.

3. Litchfield, J. T., Wilcoxon, F., *J. Pharm. Exp. Therap.*, 1949, v95, 99.

4. Fouts, J. R., Adamson, R. H., *Science,* 1959, v129, 897.

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Artificial Respiration in Mice During Thoracic Surgery: A Simple, Inexpensive Technic.* (28717)

RICHARD SIEGLER AND MARVIN A. RICH (Introduced by W. R. Bryan)
Laboratory of Cancer Research, Albert Einstein Medical Center, Philadelphia, Pa.

During experiments designed to determine the effect of unilateral thymectomy on the unilateral development of thymic lymphoma in mice(1,2) an apparatus for artificial respiration with positive pressure oxygen in mice was developed. Use of the device permits wide surgical exposure of the thorax and approximates the conditions accomplished by modern anesthesia machines. Because the device decreases the hazard of pneumothorax during experimental surgery on mice and is inexpensive to construct, the apparatus is presented for possible interest to other investigators.

The apparatus consists of 3 parts: 1) a flow valve, bubble meter, and high pressure limit assembly; 2) a valve to provide intermittent oxygen flow, and 3) an endotracheal catheter (Fig. 1.)

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