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SOME OBSERVATIONS ON THE LETHAL EFFECTS OF VARIOUS CHEMICALS AGAINST THE FREE-LIVING STAGES OF SCLEROSTOMES (NEMATODA)*

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After the discovery of the life history of the hookworms of man, control measures included the treatment of night-soil with various readily available chemicals, such as sodium chloride, ammonium sulphate, sodium nitrate, other fertilizers, and with urine to kill the eggs and free-feeding larvae.

In 1933 work was begun at the Institute of Parasitology of McGill University† with a view to finding chemicals which could be used to kill the free-living stages of Sclerostomes in manure heaps. It was soon found that this only dealt with one aspect of the problem, and left the control of larvae in stables and sheds and on pastures unsolved; just as treating night-soil leaves the problem of larvae on the wood and metal of latrines, and faeces in the fields as sources of hookworm infection. For these reasons a much wider range of chemicals was tested. It was also suggested that information on the types of chemicals which are lethal to the free-living stages of bursate nematodes might suggest types of chemicals which would be lethal to some of the nematodes which are parasitic on plants.

In 1946 the work was continued at the Department of Zoology of Edinburgh University.‡ The range of chemicals tested was again considerably widened, and included many which were of only theoretical interest, in order to test some theories which had been formulated on the chemical constitution of substances which are lethal. For this purpose appropriate chemicals were made at the Heriot-Watt College.‡ Furthermore it was hoped that indications might be obtained on the types of chemical which could be used as anthelmintics; especially as anthelmintics against the immature forms of bursate nematodes in the abomasum and small intestine. At present there is no satisfactory anthelmintic against many immature nematodes, a want which constitutes a very serious deficiency in prophylactic dosing of farm animals.

Wright and Schaffer (1932) tested a series of chlorinated aliphatic hydrocarbons against hookworms in dogs and found that anthelmintic effect depended upon solubility in water, length of carbon chain, and the position of the halogen in the molecule. During recent years many chemicals have been tested as anthelmintics in laboratory animals and against plant nematodes. In addition, numerous chemicals

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have been tested *in vitro* by Lamson and Brown (1936) and Baldwin (1943, 1948) with *Ascaris lumbricoides,* by Chance and Mansour (1949) with *Fasciola hepatica*, and by Duguid and Heathcote (1950) with *Moniezia expansa.* These screening tests have given many indications of the types of chemicals which are lethal to various adult helminths. Levine (1949, 1951a and b) screened numerous chemicals against Sclerostome larvae in faeces, from which he was able to draw some conclusions on the types of chemicals which are lethal to the free-living stages of bursate nematodes.

However, at present there is no thoroughly satisfactory anthelmintic for some of the common bursate nematodes of domestic animals; there is no chemical which could be recommended to kill bursate larvae in latrines, stables, sheds, or pens, and there is no practical method of killing larvae on pastures.

The results given in this paper can almost be considered as *in vivo* tests against the free-living stages of bursate nematodes, and, in some respects, as *in vitro* anthelmintic tests against the immature parasitic forms of some genera. The fact that the tests of the chemicals as ovicides and larvicides were in small cultures in a confined space at a comparatively high temperature (25° C.) gave the chemicals greater opportunities of being effective than would occur in practice.

METHOD

Chemicals have been tested by adding them to 40 g. of horse faeces within five hours of the faeces being passed. When the chemicals were tested in the dry state, they and the faeces were mixed and tied up in butter muslin or cheese cloth, and put into glass preserving jars of about 550 c.c. capacity, fitted with lids without rubber rings. Chemicals in solution or suspension were poured slowly over the faeces which had previously been tied up in butter muslin and placed in the jars. A few chemicals were tested in 80, 16, and 10 g. cultures, contained in proportionately sized jars; the smaller jars were fitted with shives with a small hole bored in them. The cultures were kept for a minimum of ten days, but usually longer, in a constant temperature room, at $25 \pm 2°$ C., which gave the free-living stages more than sufficient time to reach the infective stage. Untreated control cultures were always made. The larvae, if any, were extracted by soaking the cultures in warm water in 8-in. glass funnels. To collect any larvae on the walls of the jars, they were filled with water, which was also poured into the funnels. Two or three days later more warm water was added to the funnels. Six to ten days later the bottom 50 c.c. of each funnel was drawn off into an Erlenmeyer flask. The larvae in this fluid were then counted on a squared glass dish with the help of a dissecting microscope; if the larvae were numerous a dilution technique was used before counting—control cultures usually contained several thousand larvae. This technique has already been described in greater detail by Parnell (1936, 1938).

When a chemical was first tested cultures were made in a wide range of concentrations. After these preliminary cultures had shown the probable quantity of chemical required to kill the free-living stages, cultures were made in duplicate above, at, and below the indicated lethal quantities; and single cultures were made with still greater and lesser amounts of chemical. The final results, therefore, were usually based on three cultures for the quantities of chemical approximating to the lethal percentage, and in addition there were series of one or two cultures with considerably more and considerably less chemical.

The majority of the chemicals were tested by being mixed dry or undiluted in the faeces, and, if soluble in water, were also tested as aqueous solutions; a few chemicals were tested as aqueous suspensions. Amounts up to 20 g. of solid and of 25 c.c. of liquid were added to 40 g. of faeces; 25 c.c. of fluid is about the maximum quantity that 40 g. of fresh horse faeces will absorb. The more effective the chemicals were the more accurately were their values assessed. When very small amounts of chemical were applied the difference between quantities was only 0.001 g. or 0.001 c.c., but with larger quantities the difference was as much as 4 g. or 5 c.c. Therefore, it follows that with the less lethal chemicals smaller percentages might have been effective. Similarly, if more than three cultures had been made, sometimes the arbitrary 90 and 99.9 reduction percentages might have been slightly different; this is especially true where some larvae avoided the action of the chemical in one culture.

In the following Tables, " very concentrated solutions " implies 1 g. of chemical to 2 or 4 c.c. of water, " concentrated solutions " were 1 g. of chemical to 8 or 20 c.c. of water, " moderately concentrated solutions " were 1 g. of chemical to 50 or 100 c.c. of water, " moderately dilute solutions " were 1 g. of chemical to 200, 300, 400, or 500 c.c. of water, " dilute solutions " were 1 g. of chemical to 600, 800, 1,000, 1,250, and 1,500 c.c. of water, " very dilute solutions " were 1 g. of chemical to 2,000, 2,500, 3,000, 4,000, 5,000, 6,000, and 8,000 c.c. of water, and " extremely dilute solutions " were 1 g. of chemical to 10,000, or more, c.c. of water. The most effective results are quoted for each group of solutions. A few chemicals were also measured as gases.

The description " third stage larvae " has been used in most places in this paper in preference to infective larvae, because it is probable that some of the larvae which reached the third stage in treated cultures, although alive and often active, had been damaged sufficiently by the chemical to reduce considerably their ability to infest a host.

Results

Table I gives the percentages by weight of eighty inorganic chemicals and Table II gives similar figures for nearly two hundred organic chemicals and other substances which gave a 90 and a 99.9 per cent reduction in the number of third stage larvae found in the cultures. Forty-five chemicals and other materials are listed which have no lethal value or such a low value that it was not determined. Reductions in the percentage of larvae recovered caused by alteration in the consistency of the cultures have been ignored, as far as possible. Sometimes insufficient chemical was added to the cultures to enable the lethal value to be determined; this is indicated in Tables I, II, and III by an asterisk (*); where the quantity of solid or undiluted chemical was more or less than 50 per cent it is shown by including the percentage in brackets below the asterisk. When the chemical was in solution the maximum amount applied has been the percentage in 25 c.c. added to 40 g. faeces.

Only the most lethal chemicals were tested as very dilute or extremely dilute solutions; that is, in solutions containing only one gramme of chemical in 2,000 c.c., or more, of water. Table III lists those chemicals which were effective in " very and extremely dilute solutions," and shows the percentages of chemical to faeces required for 90 and 99.9 per cent reductions in the average numbers of third stage larvae recovered.

Although free-feeding larvae are usually more easily killed by unfavourable conditions than are infective larvae, some of the larvae have been able to develop to the third stage before being killed in cultures treated with some chemicals. A few of these chemicals have killed all these larvae, others have only killed some. Chemicals which fairly frequently showed this delayed killing action are listed in Table IV. Often the percentage of chemical necessary to produce this phenomenon varied considerably, depending on whether the chemical was added as a solid or

TABLE I

INORGANIC CHEMICALS

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by											
	90% when applied						99.9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Sodium compounds:												
Sodium hydroxide	1.9	3.0	1.9	1.25	—	—	2.5	4.0	2.5	—	—	—
Sodium bicarbonate	7.5	—	6.0	—	—	—	20.0	—	7.5	—	—	—
Sodium fluoride	0.5	—	0.9	0.2	0.08	0.06	0.8	—	0.9	0.4	0.25	*
Sodium chloride	2.5	4.1	1.9	*	—	—	5.0	4.1	2.5	*	—	—
Sodium iodide	0.02	0.2	0.12	0.1	0.025	0.02	0.19	0.9	0.37	0.13	0.05	0.04
An aqueous solution of sodium hypochlorite with 1.2% available chlorine	19.0	*	*	—	—	—	25.0	*	*	—	—	—
Sodium chlorate	3.75	4.0	2.5	—	—	—	6.25	5.75	6.0	—	—	—
Sodium sulphate	12.5	14.2	*	—	—	—	20.0	19.0	*	—	—	—
Sodium sulphite	10.0	8.6	*	—	—	—	15.0	25.0	*	—	—	—
Sodium sulphide	10.0	7.2	7.4	—	—	—	12.5	14.4	*	—	—	—
Sodium nitrate	3.7	2.9	2.3	—	—	—	6.2	5.8	3.1	—	—	—
Trisodium phosphate	6.2	7.2	6.0	—	—	—	12.25	12.5	*	—	—	—
Sodium tetraborate	0.63	—	0.9	0.4	—	—	3.75	—	1.8	1.0	—	—
Sodium silicofluoride	1.25	—	—	0.3	0.06	0.06	3.7	—	—	0.5	0.2	*
Potassium compounds:												
Potassium hydroxide	2.5	3.0	2.5	*	—	—	3.75	4.6	4.5	*	—	—
Potassium carbonate	7.5	5.0	4.5	—	—	—	7.5	7.5	7.5	—	—	—
"Muriate of potash," fertilizer	3.7	4.3	3.7	—	—	—	7.5	5.7	6.0	—	—	—
Potassium iodide	0.025	0.6	0.3	0.13	0.04	0.015	0.075	1.2	0.5	0.25	0.06	0.05
Potassium chlorate	15.0	6.2	3.1	*	—	—	35.0	15.5	6.0	*	—	—
Potassium iodate	0.05	—	0.25	0.1	0.05	0.025	0.62	—	0.5	0.19	0.08	0.08
Potassium sulphate	10.0	11.5	7.5	—	—	—	30.0	20.0	*	—	—	—
Potassium nitrate	3.7	4.3	3.0	—	—	—	12.5	4.3	4.5	—	—	—
Potassium cyanate	1.25	1.15	0.9	0.5	*	—	1.9	1.5	1.8	0.75	*	—
162 parts potassium cyanate + 96 parts ammonium carbonate	1.25	1.5	1.25	0.5	—	—	1.9	1.5	1.6	*	—	—
Potassium permanganate ..	7.5	—	*	—	—	—	25.0	—	*	—	—	—
Ammonia and ammonium compounds:												
Aqueous ammonia (containing 27% NH_3)	1.3	1.5	2.1	*	—	—	1.3	2.1	2.1	*	—	—
Ammonium carbonate	1.9	1.6	1.4	1.0	—	—	2.5	1.9	1.8	*	—	—
Ammonium chloride	1.9	2.1	1.1	1.2	—	—	3.7	4.0	1.1	*	—	—
Ammonium iodide	0.08	0.44	0.19	0.1	0.033	0.024	0.5	1.8	0.5	0.19	0.08	0.09
Ammonium sulphate	5.0	4.3	3.0	—	—	—	10.0	7.2	6.0	—	—	—
Ammonium sulphide (15% aqueous solution)	6.2	4.2	5.5	—	—	—	10.0	6.2	7.0	—	—	—
Ammonium sulphamate ..	0.82	1.5	0.6	0.5	0.2	—	2.5	2.4	1.85	0.62	*	—
Ammonium nitrate	1.9	3.9	1.9	—	—	—	3.7	4.0	3.0	—	—	—
"Nitro chalk"	6.2	5.0	3.1	—	—	—	7.5	7.2	7.5	—	—	—
"Calnitro"	2.5	4.3	2.5	—	—	—	6.2	7.2	6.0	—	—	—
Diammonium phosphate ..	3.7	4.3	2.5	—	—	—	6.2	7.2	6.0	—	—	—
Ammonium thiocyanate ..	0.5	0.8	0.6	0.2	0.13	—	1.9	2.1	1.5	0.5	*	—
Copper compounds:												
Cuprous chloride	3.75	—	—	—	—	—	6.2	—	—	—	—	—
Cupric chloride	1.9	2.5	1.6	0.6	—	—	3.75	3.1	3.1	*	—	—
Cupric sulphate	6.2	4.3	4.5	*	—	—	15.0	8.6	*	*	—	—
Cupric nitrate	3.7	2.5	1.6	1.2	—	—	6.2	4.2	3.1	*	—	—
Magnesium compounds:												
Magnesium chloride	6.2	7.2	6.0	—	—	—	12.5	8.6	7.5	—	—	—
Magnesium sulphate	17.5	15.5	*	—	—	—	35.0	*	*	—	—	—
Kainite (high grade)	3.7	2.9	2.2	—	—	—	5.0	4.0	7.5	—	—	—
Magnesium borate	0.5	—	—	—	—	—	1.9	—	—	—	—	—

TABLE I—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied						99·9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Calcium compounds:												
Bleaching powder	5.0	4.3	3.5	—	—	—	7.5	7.5	5.5	—	—	—
Calcium nitrate	3.7	4.3	3.0	—	—	—	10.0	5.7	7.5	—	—	—
"Calurea"	1.2	0.9	1.2	1.2	—	—	3.7	1.4	1.2	1.2	—	—
16% superphosphate	35.0	—	—	—	—	—	50.0	—	—	—	—	—
20% superphosphate	15.0	—	—	—	—	—	20.0	—	—	—	—	—
Calcium arsenate	1.0	—	—	—	—	—	20.0	—	—	—	—	—
Calcium cyanide	0.05	—	—	—	—	—	0.19	—	—	—	—	—
Calcium cyanamide (powdered)	1.9	1.8	0.6	0.8	—	—	1.9	4.8	1.8	*	—	—
Calcium cyanamide (granular)	1.2	—	—	—	—	—	2.5	—	—	—	—	—
Calcium chloroacetate	1.25	1.15	0.9	0.25	0.25	—	3.75	5.75	1.85	1.0	*	—
Zinc compounds:												
Zinc chloride	1.9	1.6	1.25	0.5	—	—	3.75	4.2	1.9	1.0	—	—
Zinc iodide	0.13	0.35	0.19	0.06	0.02	0.02	0.63	1.0	0.3	0.13	0.05	0.05
Zinc sulphate	1.3	2.5	1.25	0.5	—	—	15.0	5.8	3.1	*	—	—
Zinc sulphide	50.0	—	—	—	—	—	*	—	—	—	—	—
Mercury compounds:												
Mercurous chloride	0.037	—	—	—	—	—	0.1	—	—	—	—	—
Mercuric chloride	0.025	—	0.025	0.008	0.008	0.009	0.062	—	0.1	0.025	0.019	0.017
Ethyl-mercuric chloride	0.004	—	—	—	—	—	0.025	—	—	—	—	—
Ethoxy-ethyl-mercuric chloride	0.025	—	—	—	0.006	0.008	0.37	—	—	—	0.04	0.03
Mercuric iodide	0.12	—	—	—	—	—	3.7	—	—	—	—	—
A seed dressing containing mercury alkoxy-ethyl	1.25	—	—	—	—	—	2.5	—	—	—	—	—
Boron compound:												
Ortho-boric acid	0.63	—	—	—	—	—	1.9	—	—	—	—	—
Carbon compound:												
Carbon disulphide	0.32	—	—	—	—	—	1.6	—	—	—	—	—
Iodine:												
Iodine	0.08	—	—	—	—	—	0.38	—	—	—	—	—
10% colloidal iodine in vegetable oil	0.8	—	—	—	—	—	7.5	—	—	—	—	—
0.12% iodine in oily medium	32.0	—	—	*	—	—	62.0	—	—	*	—	—
"Iodine vermicide"—containing 16% iodine	1.9	—	0.3	0.4	0.08	*	5.0	—	0.08	0.8	0.3	*
Manganese compounds:												
Manganous chloride	2.5	3.1	2.25	1.25	—	—	6.2	6.2	3.0	*	—	—
Manganous sulphate	7.5	4.0	1.5	—	—	—	12.5	8.5	2.5	—	—	—
Iron compounds:												
Ferrous chloride	3.75	2.5	1.85	*	—	—	3.75	5.0	3.1	*	—	—
Ferrous iodide	0.08	—	—	0.12	0.06	0.05	0.8	—	—	0.38	0.1	*
Ferrous sulphate	3.75	4.0	2.5	—	—	—	10.0	7.0	6.0	—	—	—
Ferric chloride	3.75	2.3	2.1	*	—	—	3.75	2.9	3.1	*	—	—
Ferric sulphate	3.7	—	—	—	—	—	5.0	—	—	—	—	—
Cobalt compound:												
Cobalt chloride	3.75	2.9	1.85	0.5	—	—	6.25	5.7	2.45	*	—	—
Nickel compound:												
Nickel chloride	3.75	2.8	1.25	0.5	0.17	—	10.0	5.8	2.5	1.25	0.3	—

TABLE II

ORGANIC CHEMICALS

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied: Dry or undiluted	90%: In very concentrated solutions	90%: In concentrated solutions	90%: In moderately concentrated solutions	90%: In moderately dilute solutions	90%: In dilute solutions	99.9% when applied: Dry or undiluted	99.9%: In very concentrated solutions	99.9%: In concentrated solutions	99.9%: In moderately concentrated solutions	99.9%: In moderately dilute solutions	99.9%: In dilute solutions
ALIPHATIC COMPOUNDS												
Paraffins:												
44.3% Naphtha +5.3% oleic acid 1.5% Teepol X 0.6% NaOH and water ..	0.23	—	0.2	0.23	0.23	—	0.35	—	0.44	0.34	0.29	—
Halogen derivatives of paraffins:												
Methyl bromide	0.009	—	—	—	—	—	0.02	—	—	—	—	—
Methyl iodide	0.006	—	—	0.006	0.004	0.003	0.012	—	—	0.012	0.007	0.007
Ethyl chloride	4.5	—	—	—	*	—	*(57.5)	—	—	—	—	*
Ethyl bromide	0.15	—	—	—	—	—	0.4	—	—	—	—	—
Ethyl iodide	0.02	—	—	—	0.024	0.015	0.07	—	—	—	0.035	0.04
n-Propyl bromide	0.11	—	—	—	*	—	0.25	—	—	—	*	—
n-Propyl iodide	0.033	—	—	—	—	0.03	0.066	—	—	—	—	0.09
n-Butyl chloride	0.74	—	—	—	—	—	1.12	—	—	—	—	—
n-Butyl bromide	0.1	—	—	—	—	—	0.32	—	—	—	—	—
tert.-Butyl bromide	1.2	—	—	—	—	—	1.5	—	—	—	—	—
n-Amyl bromide	0.3	—	—	—	—	—	0.6	—	—	—	—	—
n-Hexyl bromide	0.072	—	—	—	—	—	0.16	—	—	—	—	—
n-Heptyl bromide	0.043	—	—	—	—	—	0.14	—	—	—	—	—
n-Octyl bromide	0.055	—	—	—	—	—	0.14	—	—	—	—	—
Methylene dichloride	0.67	—	—	—	—	—	2.5	—	—	*	—	—
Methylene dibromide	0.47	—	—	0.77	0.5	—	0.63	—	—	1.2	0.8	—
Methylene diiodide	0.008	—	—	0.04	0.012	0.004	0.02	—	—	0.23	0.07	0.03
Ethylene dichloride	0.5	—	—	*	*	—	1.0	—	—	*	*	—
Ethylidene dichloride	1.17	—	—	—	*	—	2.2	—	—	—	*	—
10% methyl bromide + 67.5% ethylene dichloride, 22.5% carbon tetrachloride	0.47	—	—	—	—	—	0.78	—	—	—	—	—
Ethylene dibromide	0.08	—	—	—	—	—	0.08	—	—	—	—	—
1:3-Dichloropropylene + 1:2-Dichloropropane + higher chlorides (D.D. mixture) ..	0.01	—	—	—	—	—	0.019	—	—	—	—	—
Dichloropentanes	0.27	—	—	—	—	—	0.54	—	—	—	—	—
Chloroform	1.2	—	—	—	*	—	3.75	—	—	—	*	—
Bromoform	0.18	—	—	—	0.22	0.15	0.29	—	—	—	0.36	0.22
Iodoform	0.075	—	—	—	—	—	0.18	—	—	—	—	—
Chloropicrin	0.02	—	—	—	—	—	0.02	—	—	—	—	—
1:1:1-Trichloroethane	1.1	—	—	—	—	—	2.5	—	—	—	—	—
Carbon tetrachloride	1.3	—	—	—	—	—	5.9	—	—	—	—	—
Carbon tetrabromide	0.019	—	—	—	—	—	0.025	—	—	—	—	—
1:1:2:2-Tetrachloroethane ..	0.1	—	—	—	—	—	0.13	—	—	—	—	—
Pentachloroethane	0.21	—	—	—	—	—	0.42	—	—	—	—	—
Hexachloroethane	0.12	—	—	—	—	—	0.25	—	—	—	—	—
Halogen derivatives of ethylene:												
Dichlorethylene (*cis*)	0.65	—	—	—	—	—	1.06	—	—	—	—	—
Dichlorethylene (*trans*)	0.62	—	—	—	—	—	1.56	—	—	—	—	—
Trichlorethylene	0.36	—	—	—	—	*	0.72	—	—	—	—	*
Perchloroethylene	0.31	—	—	—	—	—	0.31	—	—	—	—	—
Alcohols:												
Methyl alcohol	4.0	2.65	3.4	—	—	—	5.0	6.0	*	—	—	—
Ethyl alcohol	3.0	2.0	2.8	—	—	—	5.0	3.3	4.4	—	—	—
n-Propyl alcohol	1.5	1.3	1.7	*	—	—	1.5	1.6	2.2	*	—	—
iso-Propyl alcohol	1.5	1.6	1.6	*	—	—	3.0	2.0	2.3	*	—	—
n-Butyl alcohol	0.66	—	0.95	0.8	—	—	1.5	—	1.7	1.0	—	—
iso-Butyl alcohol	0.8	—	0.9	0.97	—	—	3.0	—	1.1	*	—	—
tert.-Butyl alcohol	1.5	1.0	1.1	*	—	—	1.5	1.3	1.6	*	—	—
Ethers:												
Ether	1.8	—	1.3	—	—	—	3.6	—	*	—	—	—
Dichlorodiethyl ether	0.06	—	—	0.05	0.05	0.05	0.1	—	—	0.09	0.11	0.11
50% β-butoxy-β-thiocyanodiethyl ether + 50% petroleum distillate of the kerosene type	0.46	—	—	—	—	—	0.75	—	—	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied						99.9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Sulphides:												
Tetramethyl thiuram monosulphide	1.9	—	0.9	0.25	0.19	—	40.0	—	1.9	1.25	*	—
46% tetramethyl thiuram disulphide	5.0	—	0.9	0.2	0.06	0.1	7.5	—	1.8	0.6	0.16	*
Formaldehyde and derivative:												
Formalin	2.0	0.7	0.5	0.27	0.2	—	2.0	1.4	1.2	0.55	0.35	—
Hexamethylene tetramine ..	7.5	4.5	*	—	—	—	12.5	18.0	*	—	—	—
Ketone:												
Acetone	3.6	3.3	4.4	—	—	—	8.0	6.6	5.5	—	—	—
Soaps:												
Hard soap (Castile)	—	11.0	—	—	—	—	—	*	—	—	—	—
Soft soap	—	*	—	—	—	—	—	*	—	—	—	—
Esters:												
Ethyl iodoacetate	0.06	—	—	—	—	—	0.19	—	—	—	—	—
Dodecyl thiocyanate	3.75	—	—	—	—	—	60.0	—	—	—	—	—
50% mixed thiocyanates + 50% petroleum distillate of the kerosene type	0.46	—	—	—	—	—	0.75	—	—	—	—	—
Hexaethyl tetraphosphate ..	3.8	—	—	*	—	—	6.2	—	—	*	—	—
Di-ethyl-*p*-nitrophenylthiophosphate	0.8	—	—	—	—	—	39.0	—	—	—	—	—
Sodium alkyl sulphates (Teepol X)	10.5	—	1.6	*	—	—	66.0	—	*	*	—	—
Amines:												
25% dimethylamine	3.5	1.9	2.7	*	—	—	3.5	3.1	5.2	*	—	—
Cetyltrimethylammonium bromide	3.75	—	1.8	0.75	—	—	30.0	—	*	*	—	—
20% solution of alkyl ammonium bromides	—	—	1.5	—	—	—	—	—	*	—	—	—
10% alkyl-dimethyl-benzyl ammonium chlorides	25.0	6.2	2.4	0.6	—	—	37.5	*	*	*	—	—
Allyl compounds:												
Allyl isothiocyanate	0.0015	—	—	—	0.002	0.002	0.0025	—	—	—	0.004	0.003
Allyl chloride	0.1	—	—	—	—	—	0.23	—	—	—	—	—
Allyl bromide	0.007	—	—	—	—	—	0.026	—	—	—	—	—
Allyl iodide	0.0012	—	—	—	—	—	0.003	—	—	—	—	—
Allyl alcohol	0.05	0.05	0.05	0.03	0.05	0.07	0.16	0.08	0.1	0.08	0.11	*
Dihydric alcohol and derivative:												
2-Ethyl-hexanediol-1 : 3	10.0	—	—	—	—	—	19.0	—	—	—	—	—
" Lubrol W."	—	—	1.0	1.0	—	—	—	—	*	*	—	—
Keto-acid:												
Pyruvic acid	6.3	3.1	1.9	1.55	—	—	15.7	5.3	3.0	*	—	—
Carbonic acid derivatives:												
80% zinc dimethyldithiocarbamate	*	—	1.5	0.4	0.16	—	*	—	3.1	0.6	*	—
80% ferric dimethyldithiocarbamate	1.25	—	0.9	0.4	0.2	—	*	—	2.4	0.6	*	—
Urea	0.8	0.7	0.6	0.5	—	—	0.8	0.7	0.9	0.6	—	—
Guanidine hydrochloride ..	—	—	0.9	0.3	—	—	—	—	1.5	0.6	—	—
Methylguanidine sulphate ..	—	—	0.9	0.5	—	—	—	—	1.8	1.2	—	—
as-Dimethylguanidine sulphate..	—	—	0.9	0.5	—	—	—	—	1.5	1.0	—	—
Diphenylguanidine	—	—	2.5	0.6	—	—	—	—	*	*	—	—
Creatine	—	—	—	0.9	—	—	—	—	—	*	—	—
Creatinine	—	—	0.9	1.0	—	—	—	—	*	1.2	—	—
Thiourea	0.1	—	0.3	0.1	0.06	0.06	0.4	—	1.8	0.3	0.2	*
Potassium xanthogenate ..	0.5	0.7	0.9	0.2	0.16	—	1.9	2.4	1.2	0.6	*	—
Esters of dibasic acids:												
Butyl mesityl oxalic ester ..	10.4	—	—	—	—	—	13.0	—	—	—	—	—
Butyl mesityl oxide oxalate ..	19.0	—	—	—	—	—	38.0	—	—	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by											
	90% when applied						99.9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Ureides and purine derivatives:												
Barbituric acid	3.75	—	—	1.25	*	—	17.5	—	—	*	*	—
Allantoin	1.25	—	—	—	—	—	*	—	—	—	—	—
Xanthine	7.5	—	—	—	*	—	* (10.0)	—	—	—	*	—
Guanine	10.0	—	—	—	—	—	* (15.0)	—	—	—	—	—
CARBOCYCLIC COMPOUNDS												
Hydrocarbons:												
Benzene	0.56	—	—	—	—	—	0.72	—	—	—	—	—
Toluene	0.16	—	—	—	—	—	0.86	—	—	—	—	—
Xylenes	0.09	—	—	—	—	—	0.16	—	—	—	—	—
p-Cymene	0.09	—	—	—	—	—	0.16	—	—	—	—	—
Halogen derivatives:												
Chlorobenzene	0.11	—	—	—	—	—	0.2	—	—	—	—	—
Bromobenzene	0.09	—	—	—	—	—	0.12	—	—	—	—	—
Iodobenzene	0.11	—	—	—	—	—	0.18	—	—	—	—	—
o-Dichlorobenzene	0.11	—	—	—	—	—	0.24	—	—	—	—	—
p-Dichlorobenzene	0.12	—	—	—	—	—	0.37	—	—	—	—	—
2:3:5:6-Tetrachloronitrobenzene	17.5	—	—	—	—	—	*	—	—	—	—	—
Cyclohexyl bromide	0.16	—	—	—	—	—	0.32	—	—	—	—	—
Mercury compounds:												
Phenyl mercuri-acetate	0.01	—	—	—	—	—	0.12	—	—	—	—	—
An organic mercurial seed dressing containing 1% mercury ..	0.8	—	—	—	—	—	3.75	—	—	—	—	—
Amino-compounds:												
Aniline	0.04	—	—	0.06	0.05	0.03	0.12	—	—	0.13	0.13	0.13
p-Aminophenyl arsonic acid ..	1.25	—	—	0.6	—	—	2.5	—	—	*	—	—
Diphenylamine	12.5	—	—	—	—	*	*	—	—	—	—	*
Diphenylamine chloroarsine ..	10.0	—	—	—	—	—	20.0	—	—	—	—	—
Azo-compounds:												
Azobenzene	1.25	—	—	—	—	—	2.5	—	—	—	—	—
Amino-azobenzene-hydrochloride	10.0	—	—	—	—	—	*	—	—	—	—	—
Phenols:												
Phenol	0.4	0.3	0.3	0.2	0.11	0.11	1.3	1.9	0.5	0.3	0.3	*
40% low boiling phenols in emulsion	0.8	1.0	0.4	0.3	0.3	—	2.6	5.1	0.9	1.0	*	—
40% high boiling phenols in emulsion	5.0	2.5	1.2	0.5	—	—	12.5	5.0	1.5	1.2	—	—
60% high boiling phenolic bodies in emulsion	18.75	3.75	1.8	0.75	—	—	31.0	10.0	2.4	*	—	—
Sodium 2:4:5-trichlorophenate	0.5	2.0	0.9	0.1	0.06	—	1.25	4.3	1.6	0.5	*	—
o-Iodo-phenol	—	—	—	0.3	0.1	—	—	—	—	0.5	0.3	—
o-Nitro-phenol	0.25	—	—	—	—	—	0.25	—	—	—	—	—
p-Nitro-phenol	1.25	—	—	0.13	0.1	—	5.0	—	—	0.6	0.25	—
Sodium *o*-phenyl phenate ..	5.0	5.25	0.5	0.25	0.1	—	10.0	6.6	3.0	0.6	0.25	—
Sodium *m*-chloro-*p*-phenyl-phenate	10.0	7.2	1.5	0.25	0.13	—	17.5	14.3	*	0.6	*	—
2-Cyclohexyl cyclohexanol + 2-phenyl cyclohexanol	4.7	—	—	—	—	—	7.1	—	—	—	—	—
50% cresol	3.7	1.0	0.9	0.4	0.2	—	7.5	2.5	1.5	0.6	*	—
Monochloroxylenols with some dichloroxylenols	0.5	—	—	—	—	—	3.8	—	—	—	—	—
2:4-Dichloro-*sym*.-xylenol ..	1.9	—	—	—	—	—	20.0	—	—	—	—	—
"Dettol" (halogenated xylenol in aromatic oils)	1.0	1.25	0.6	0.5	—	—	2.5	3.3	1.8	1.0	—	—
Thymol	1.0	—	—	—	—	—	1.9	—	—	—	—	—
Resorcinol	0.25	1.0	0.6	0.13	0.08	0.09	1.9	5.0	1.5	1.0	*	*
4-*n*-Hexylresorcinol	6.2	—	—	—	—	—	*	—	—	—	—	—
Ketone:												
p-Chloro-acetophenone	1.25	—	—	—	—	—	2.5	—	—	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied: Dry or undiluted	90%: In very concentrated solutions	90%: In concentrated solutions	90%: In moderately concentrated solutions	90%: In moderately dilute solutions	90%: In dilute solutions	99.9% when applied: Dry or undiluted	99.9%: In very concentrated solutions	99.9%: In concentrated solutions	99.9%: In moderately concentrated solutions	99.9%: In moderately dilute solutions	99.9%: In dilute solutions
Monobasic acids and derivatives:												
Benzoic acid	17.5	—	—	—	*	—	*	—	—	—	*	—
Benzyl benzoate	28.0	—	—	—	—	—	* (70.0)	—	—	—	—	—
Chloromethylphenoxy-acetic acid	18.7	—	*	*	—	—	31.0	—	*	*	—	—
1% trichlorophenylmethyliodo-salicylate	19.0	8.3	7.0	—	—	—	25.0	16.6	*	—	—	—
Ester of dibasic acid:												
Dimethyl phthalate	11.9	—	—	—	—	—	45.0	—	—	—	—	—
Triphenylmethane dyes:												
Malachite green	3.75	—	—	1.0	—	—	12.5	—	—	*	—	—
Brilliant green	7.5	—	3.1	1.25	—	—	*	—	*	*	—	—
Magenta	50.0	—	*	*	—	—	*	—	*	*	—	—
Methyl violet	30.0	—	—	*	—	—	*	—	—	*	—	—
Gentian violet	35.0	—	3.1	1.25	—	—	*	—	*	*	—	—
Methyl green	7.5	—	2.5	1.0	—	—	17.5	—	*	*	—	—
Terpenes:												
Turpentine	0.55	—	—	—	—	—	1.3	—	—	—	—	—
Essential oil ex *Artemisia maritima* containing 65% β-thujone and 16% 1 : 8-cineole	0.1	—	—	—	—	—	0.17	—	—	—	—	—
10% of essential oil of *Artemisia maritima* in an aqueous alcoholic soap basis	0.74	0.75	0.75	0.5	*	—	0.91	1.1	1.3	1.1	*	—
β-Thujone	0.092	—	—	—	—	—	0.092	—	—	—	—	—
1 : 8-Cineole	0.23	—	—	—	—	—	0.58	—	—	—	—	—
Oil of chenopodium	0.1	—	—	—	—	—	0.25	—	—	—	—	—
5% oil of chenopodium with 95% castor oil	2.5	—	—	—	—	—	6.2	—	—	—	—	
Naphthalene and derivative:												
Naphthalene	0.19	—	—	—	—	—	0.38	—	—	—	—	—
β-Naphthol	12.5	—	—	—	—	—	*	—	—	—	—	—
Saponins:												
Saponin mixture	20.0	25.0	6.0	—	—	—	*	*	*	—	—	—
HETEROCYCLIC COMPOUNDS												
Benzthiazole:												
2-Mercaptobenzthiazole	35.0	—	—	—	—	—	*	—	—	—	—	—
Pyridine and derivatives:												
Pyridine	0.19	0.16	0.18	0.19	0.19	—	0.25	0.25	0.24	0.25	0.25	—
α-Picoline	0.09	0.07	0.085	0.07	0.09	0.1	0.17	0.12	0.17	0.14	0.12	*
β-Picoline	0.18	0.12	0.11	0.14	0.15	—	0.18	0.16	0.17	0.23	0.23	—
γ-Picoline	0.18	0.12	0.11	0.14	0.12	—	0.18	0.16	0.17	0.23	0.24	—
2 : 6-Lutidine	0.09	0.07	0.05	0.07	0.08	0.08	0.17	0.12	0.08	0.09	0.12	*
32.9% β-Picoline+16.9% γ-picoline+50.2% 2 : 6-lutidine	0.23	0.12	0.1	0.12	0.12	—	0.23	0.15	0.13	0.23	0.15	—
2 : 4-Lutidine+2 : 5-lutidine	0.1	—	0.065	0.07	0.06	0.06	0.18	—	0.12	0.14	0.1	0.1
2 : 4 : 6-Collidine	0.17	—	—	0.11	0.06	0.09	0.23	—	—	0.17	0.12	*
3 : 5-Lutidine+2 : 3 : 6-collidine+2 : 4 : 6-collidine	0.12	—	—	0.09	0.05	0.05	0.24	—	—	0.19	0.15	0.08
2 : 4 : 5-Collidine+*o*-toluidine+*p*-toluidine	0.16	—	—	0.11	0.06	0.05	0.34	—	—	0.17	0.11	0.1
Quinoline derivatives:												
Quinoline	0.54	—	0.52	0.27	0.09	0.11	6.9	—	1.95	0.7	0.18	*
8-Hydroxyquinoline sulphate	0.19	0.7	0.3	0.2	0.06	0.04	0.5	1.3	0.9	0.3	0.1	0.07
Acridine derivative:												
Acriflavine	2.5	—	2.5	—	—	—	6.25	—	5.6	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by											
	90% when applied						99.9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Benzthiazine derivatives:												
3-Keto-2 : 3-dihydro-benz-1 : 4-thiazine	5.0	—	—	0·5	—	—	25.0	—	—	*	—	—
3-Keto-2 : 3-dihydro-6 : 7-dimethoxy-benz-1 : 4-thiazine ..	50.0	—	—	*	—	—	*	—	—	*	—	—
Hydrochloride of 6-amino 3-keto-2 : 3-dihydro-benz-1 : 4-thiazine	7.5	—	—	*	—	—	25.0	—	—	*	—	—
Phenoxthine:												
Phenoxthine	1.25	—	—	—	—	*	*	—	—	—	—	*
Phenothiazine and derivatives:												
Phenothiazine	50.0	—	—	—	—	0.04	*	—	—	—	—	*
Mercurated phenothiazine ..	0.019	—	—	0.06	0.04	0.05	0.38	—	—	0.6	*	*
3 : 7-Dinitro-10-acetyl-phenothiazine-5-sulphoxide	50.0	—	*	—	—	—	*	—	*	—	—	—
Phenothiazine sulphoxide ..	*	—	—	0.4	—	—	*	—	—	*	—	—
Thionol hydrochloride	35.0	—	—	—	—	*	40.0	—	—	—	—	*
Phenothiazone	40.0	—	—	0.5	0.31	0.05	*	—	—	*	*	*
Lauth's violet	3.75	—	—	*	—	—	12.5	—	—	*	—	—
Lauth's violet ⇉(phenol)$_2$ ..	2.5	—	—	*	—	—	5.0	—	—	*	—	—
Lauth's violet ⇉(β-naphthol)...	5.0	—	—	*	—	—	*	—	—	*	—	—
Lauth's violet ⇉(salicylic acid)$_2$	17.5	—	—	*	—	—	35.0	—	—	*	—	—
Lauth's violet ⇉ (2-hydroxy-3-naphthoic acid)$_2$ ..	30.0	—	—	*	—	—	*	—	—	*	—	—
Lauth's violet ⇉(1-phenyl-3-methyl-5-pyrazolone)$_2$ ..	50.0	—	—	*	—	—	*	—	—	*	—	—
Brilliant alizarin	25.0	—	*	—	—	—	*	—	*	—	—	—
Methylene blue	50.0	12.0	2.5	—	—	—	*	*	*	—	—	—
Alkaloids:												
Amphetamine sulphate ..	—	—	—	0.38	0.13	—	—	—	0.75	*	—	—
40% nicotine sulphate	5.7	1.7	0.4	0.28	0.28	—	11.4	7.2	2.7	*	*	—
Tobacco dust	10.0	—	—	—	—	—	*	—	—	—	—	—
Arecoline hydrobromide ..	0.25	—	—	0.06	—	—	1.25	—	—	0.25	—	—
Quinine acid hydrochloride ..	6.25	—	*	*	—	—	17.5	—	*	*	—	—
Emetine hydrochloride	2.5	—	—	0.4	—	—	*	—	—	*	—	—
							(10.0)					
Plant product:												
Crushed flax seed	30.0	—	—	—	—	—	62.5	—	—	—	—	—
Urines and manure:												
Fresh poultry manure	40.0	—	—	—	—	—	50.0	—	—	—	—	—
Fresh horse urine	19.0	17.0	—	—	—	—	25.0	31.0	—	—	—	—
Fresh cow urine	37.5	—	—	—	—	—	60.0	—	—	—	—	—
Fresh human urine	50.0	*	—	—	—	—	*	*	—	—	—	—
Fresh dog urine	12.5	9.4	—	—	—	—	50.0	12.5	—	—	—	—

undiluted, or in strong or weak solutions. Frequently there were considerable differences in the minimum percentages of chemical required to produce this phenomenon even between solutions of the concentrated group or between solutions of the dilute group. The minimum percentages of chemical which had this effect, when applied as a solid or undiluted, or in concentrated or in dilute solutions, are shown in Table IV. Some chemicals had this effect in all or practically all the cultures; other chemicals only had this effect in some of the cultures; when this effect occurred fairly regularly the percentages in Table IV have been printed in italic type. Undoubtedly the list would have been longer if many of the chemicals had not already killed the larvae in the earlier stages. Many of these chemicals

TABLE III

The chemicals are listed which in 1 : 2,000 or in weaker solutions reduced the numbers of larvae by 90 per cent and 99.9 per cent; the percentages of chemical required to do this are also given

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by			
	90% when applied		99.9% when applied	
	In very dilute solutions	In extremely dilute solutions	In very dilute solutions	In extremely dilute solutions
Sodium iodide	0.018	—	*	—
Potassium iodide	0.016	—	*	—
Potassium iodate	0.025	—	*	—
Zinc iodide	0.016	—	*	—
Mercuric chloride	0.01	—	0.017	—
Ethoxy-ethyl-mercuric chloride	0.008	—	0.017	—
Iodine	0.02	—	*	—
Methyl iodide	0.003	0.002	0.009	0.007
Ethyl iodide	0.015	—	0.04	—
n-Propyl iodide	0.044	—	*	—
Methylene iodide	0.005	0.005	0.02	0.02
Iodoform	—	0.006	—	*
Allyl isothiocyanate	0.001	0.003	0.002	*
Phenothiazine	0.03	—	*	—

might not have killed the third stage larvae if they had not been able to damage the developing larvae.

Dilute solutions of sodium 2: 4: 5-trichlorophenate were the only dilute solutions which sometimes allowed some of the larvae to develop to the third stage before all the larvae died; the minimum concentration of this compound which did this was 0.3 g./100 c.c.

A few chemicals kill the eggs. Although this technique does not lend itself to the recovery of dead eggs, they were seen in a few of the cultures treated with potassium cyanate, 25 per cent (w/v) aqueous dimethylamine, diphenylamine, high boiling phenols, sodium 2: 4: 5-trichlorophenate, *o*-nitrophenol, sodium *o*-phenyl phenate, and sodium *m*-chloro-*p*-phenyl phenate. Less frequently dead eggs were seen in a few of the cultures treated with thiocyanate mixtures, a few of the halogen derivatives of paraffins, ethers, phenols and pyridine derivatives, and still less frequently a few eggs were seen in cultures treated with other chemicals.

A few chemicals occasionally make some of the larvae exsheath after they have reached the third stage; this was most noticeable with sodium hydroxide, sodium bicarbonate, aqueous sodium hypochlorite (1.2 per cent available chlorine), potassium hydroxide, and potassium permanganate.

Some chemicals stain the larvae and occasionally the stained larvae remain active. Chemicals which most frequently stain or darken larvae include calcium chloroacetate, ferric chloride, aminoazobenzene hydrochloride, malachite green, brilliant green, magenta, methyl violet, gentian violet, methyl green, acriflavine, phenoxthine, phenothiazone, Lauth's violet:(β-naphthol)$_2$, and methylene blue.

Occasionally *Trichostrongylus axei* larvae have been seen in these cultures; they and some of the larger larvae seem to be more resistant to some chemicals than are the smaller species of Sclerostomes.

Many chemicals and plant products have been tested which have little or no lethal effect on either the eggs or the free-living larvae. Dibutyl phthalate at 65 per cent, crushed sorgeum grass at 62.5 per cent, crushed sudan grass at 62.5 per cent, crushed tulip tree leaves at 62.5 per cent, Velsicol 1068 at 60 per cent did not produce a 90 per cent reduction in the number of larvae. The addition of 62.5 per cent of

TABLE IV

The chemicals which often allow some of the larvae to develop and then kill some or all of them are listed, with the minimum percentages of chemical which did this when applied as a solid or undiluted and in concentrated and dilute solutions. When there were dead third stage larvae in all or practically all the cultures the percentages are printed in italic type

Chemical	Minimum percentages of chemical which sometimes allow larvae to develop and then kill				
	Some			All	
	Chemical as a solid or undiluted	Chemical in concentrated solutions	Chemical in dilute solutions	Chemical as a solid or undiluted	Chemical in concentrated solutions
Sodium hydroxide	0.8	0.9	—	—	—
Sodium bicarbonate	7.5	—	—	—	—
Sodium iodide	—	0.04	0.03	—	—
Sodium tetraborate	0.63	—	—	—	—
Sodium silicofluoride	*0.25*	0.1	0.1	*0.8*	—
Potassium hydroxide	0.8	1.2	—	—	—
Potassium chlorate	5.0	1.0	—	—	—
Potassium permanganate	*1.9*	—	—	15.0	—
Ammonium thiocyanate	*0.25*	*0.2*	*0.2*	—	—
Cuprous chloride	*0.8*	—	—	*5.0*	—
Cupric chloride	*1.9*	*0.8*	—	—	1.25
Cupric sulphate	*1.9*	*1.0*	—	—	—
Cupric nitrate	*1.9*	*0.5*	—	*2.5*	*2.2*
Magnesium borate	0.38	—	—	—	—
Calcium oxide	20.0	—	—	50.0	—
Calcium hydroxide	50.0	—	—	—	—
Calurea	1.2	1.1	—	—	—
16% superphosphate	20.0	—	—	—	—
Calcium arsenate	*1.25*	—	—	—	—
Calcium chloroacetate	1.25	0.6	—	—	—
Zinc chloride	*1.25*	*0.5*	—	—	2.6
Zinc sulphate	*1.9*	*0.6*	—	—	—
Zinc sulphide	30.0	—	—	—	—
Ethyl-mercuric chloride	0.008	—	—	—	—
Ethoxy-ethyl-mercuric chloride	*0.02*	—	0.02	0.25	—
Mercuric iodide	*0.05*	—	—	1.0	—
Ferrous chloride	—	1.4	—	—	2.9
Ferrous sulphate	1.9	2.4	—	—	—
Ferric sulphate	2.5	—	—	—	—
Nickel chloride	2.5	1.7	0.1	—	—
Iodoform	0.05	—	—	—	—
Dichlorodiethyl ether	0.06	0.06	0.04	—	—
Tetramethylthiuram monosulphide	0.6	0.25	0.16	*3.75*	—
46% tetramethylthiuram disulphide	5.0	0.4	0.1	—	—
Hexamethylene tetramine	3.75	1.8	—	—	—
50% mixed thiocyanates	0.46	—	—	—	—
Hexaethyl tetraphosphate	*2.5*	—	—	—	—
Di-ethyl-*p*-nitrophenyl-thiophosphate	*0.32*	—	—	*4.7*	—
Sodium alkyl sulphates (Teepol X)	*5.25*	1.0	—	52.5	—
25% dimethylamine	2.3	1.7	—	—	—
Cetyltrimethylammonium bromide	*3.75*	1.1	—	—	—
20% solution of alkylammonium bromides	—	2.4	—	—	—
80% zinc dimethyl dithiocarbamate	*0.5*	0.4	0.2	—	—
80% ferric dimethyl dithiocarbamate	*0.6*	0.25	0.2	—	—
Guanidine hydrochloride	—	0.3	—	—	—
Methylguanidine sulphate	—	0.5	—	—	—
as-Dimethylguanidine sulphate	—	0.25	—	—	—
Diphenylguanidine	—	0.37	—	—	—
Creatine	—	0.5	—	—	—
Creatinine	—	0.9	—	—	1.85
Thiourea	0.19	0.1	0.06	—	—
Allantoin	1.0	—	—	*1.25*	—
Xanthine	*1.25*	—	—	10.0	—
Guanine	*1.0*	—	—	—	—
2:3:5:6-Tetrachloronitrobenzene	*1.9*	—	—	—	—
Benzene hexachloride	3.75	—	—	—	—
Phenyl mercuri-acetate	0.025	—	—	—	—
An organic mercurial seed dressing, containing 1% mercury	0.82	—	—	2.5	—
Aniline	0.01	—	—	—	—
Diphenylamine	*0.25*	—	—	—	—
Diphenylamine chloroarsine	6.25	—	—	—	—
Azobenzene	*0.6*	—	—	—	—
Aminoazobenzene hydrochloride	*0.6*	—	—	—	—
40% high boiling phenols in emulsion	5.0	2.0	—	—	—
Sodium 2:4:5-trichlorophenate	*0.12*	*0.03*	*0.03*	*0.8*	*0.6*
o-Iodophenol	—	0.19	0.19	—	—
p-Nitrophenol	*0.25*	0.12	—	*1.25*	—

TABLE IV—*continued*

Chemical	Minimum percentages of chemical which sometimes allow larvae to develop and then kill				
	Some			All	
	Chemical as a solid or undiluted	Chemical in concentrated solutions	Chemical in dilute solutions	Chemical as a solid or undiluted	Chemical in concentrated solutions
Sodium-*o*-phenyl phenate	*1.9*	0.3	—	*7.5*	4.1
Sodium *m*-chloro-*p*-phenyl phenate	*1.25*	*0.3*	0.2	*12.5*	*6.0*
2-Cyclohexyl cyclohexanol + 2-phenyl cyclohexanol	*1.8*	—	—	—	—
50% cresol	5.0	—	—	6.25	—
Monochloroxylenols with some dichloroxylenols	*0.2*	—	—	1.9	—
2 : 4-Dichloro-sym.-xylenol	*0.5*	—	—	*2.5*	—
4-Hexyl resorcinol	*2.5*	—	—	*7.5*	—
3 : 4-Methylenedioxy-phenyl-*n*-propyl-benzyl sulphide	2.5	—	—	—	—
p-Chloroacetophenone	*0.5*	—	—	1.25	—
Benzoic acid	*5.0*	—	—	*30.0*	—
Benzyl benzoate	*4.0*	—	—	—	—
Dimethyl phthalate	*4.5*	—	—	—	—
Malachite green	*5.0*	—	—	—	—
Brilliant green	*3.75*	—	—	—	—
Methyl violet	*7.5*	—	—	—	—
Gentian violet	*1.9*	3.1	—	—	—
Methyl green	*5.0*	—	—	—	—
β-Naphthol	*1.25*	—	—	*5.0*	—
2-Mercapto-benzthiazole	*2.5*	—	—	—	—
Quinoline	*0.27*	—	—	2.7	—
8-Hydroxyquinoline sulphate	0.19	*0.2*	0.1	—	0.8
6-Amino-3-keto-2 : 3-dihydrobenz-1 : 4-thiazine hydrochloride	5.0	—	—	—	—
Phenoxthine	*0.25*	—	0.03	—	—
Phenothiazone	*2.5*	0.5	0.06	—	—
Lauth's violet $=\gtrless(\beta\text{-naphthol})_2$	*3.75*	0.4	—	—	—
Lauth's violet $=\gtrless(\text{2-hydroxy-3-naphthoic acid})_2$	12.5	—	—	—	—
Methylene blue	30.0	—	—	—	—
Crushed sorgeum grass	50.0	—	—	—	—
Crushed sudan grass	62.5	—	—	—	—
Crushed flax seed	20.0	—	—	—	—
Fresh poultry manure	*20.0*	—	—	40.0	—

calcium oxide, of calcium hydroxide, of ground limestone, of basic slag, or of calcium phosphate (as rock phosphate) did not cause a 90 per cent reduction in the numbers of the larvae; but 20 per cent, and sometimes less, of calcium oxide and 50 per cent of calcium hydroxide killed some of the larvae after they had reached the infective stage, and 50 per cent of the former killed them all.

A 90 per cent reduction in the number of larvae was not caused by the addition to 40 g. of fresh horse faeces of 20 g. of the following substances: sublimed sulphur, ferrous sulphide, ferric oxide, cystine (but sometimes 15 per cent, or more, killed some larvae after they had developed), benzene hexachloride and its *alpha, beta, gamma, and delta* isomers separately (however, in some cultures treated with benzene hexachloride many of the larvae died after they had developed to the third stage), 1: 1: 1-trichloro-2: 2-di-*p*-chlorophenylethane (DDT), (the addition to a 44.3 per cent naphtha emulsion of 12.66 per cent of DDT, of DDD, of benzene hexachloride, or of its four isomers did not increase the lethal values of the emulsion), salicylanilide, santonin, derris powder, 6-amino-3-keto-2: 3-dihydro-benz-1: 4-thiazine, 6-chloro-3-keto-2: 3-dihydro-benz-1: 4-thiazine, 10-acetyl-phenothiazine, thionol, powdered areca nut, white hellebore powder, powdered cousso, pyrethrum powder, mowrah meal, and yeast; however, a few of these substances did kill some of the larvae after they had developed. Although 50 per cent of *m*-methoxy-*p*-hydroxy-benzaldehyde (vanillin) did not produce a 90 per cent reduction in the numbers of larvae, even small quantities caused some reduction.

The addition of 37 per cent of a 25 per cent emulsion of Filix mas reduced the number of larvae by 90 per cent, but even the addition of nearly twice as much did not give a 99.9 per cent kill; therefore much of the reduction in numbers may have been caused by the alteration in the physical condition of the cultures.

Neither 12.5 per cent of phenylethyl-*n*-octyl sulphide, of *p*-methoxyphenyl*iso*-propyl-*n*-octyl sulphide, of phenylethylbenzyl sulphide, nor of 3:4-methylenedioxy-phenyl-*n*-propylbenzyl sulphide reduced the number of larvae by 90 per cent.

Extract of quassia was only tested in a concentrated solution, in which the maximum amount applied was only 6.9 per cent of the faeces; this quantity reduced the number of larvae by about 60 per cent, a reduction which could be explained by the altered physical condition of the cultures.

The addition of 5.0 per cent of " sulphoacetamide soluble " did not produce a 90 per cent reduction of larvae, neither were 1:20 nor 1:50 aqueous solutions effective.

Lauth's violet$\rightleftarrows$(chromotropic acid)$_2$ as a 1:100 aqueous solution had little effect on the numbers of larvae.

Neither the sodium salt of penicillin, containing 100,000 units, of which over 90 per cent were penicillin G, nor an atmosphere saturated with carbon monoxide, reduced the numbers of the larvae to any appreciable extent.

Discussion

Where the difference between the amount required to kill 90 per cent and 99.9 per cent is large, it can usually be concluded that the chemical must be in intimate contact with the eggs or larvae in order to kill them. When the chemical, in contact with horse faeces, produces a lethal gas, the quantity that kills 90 per cent may also kill 99.9 per cent. However, one culture with an unusually high count of larvae can reduce the apparent lethal value of a chemical, and therefore any unexpected order of potency should, if possible, be considered in comparison with the same chemicals in a different state.

In this technique the chemicals are in the presence of a comparatively large quantity of organic matter; therefore the chemical may occasionally have been altered before the larvae hatched from the eggs. Furthermore at 25° C. a few chemicals may volatilize before the eggs have made much development.

With most compounds, whether applied solid or undiluted or in solutions, there is generally a fairly close parallelism in the order of potency for a 90 per cent and for a 99.9 per cent kill. Brief notes on the many types of chemical tested are given below.

Inorganic compounds

Data for these chemicals are given in Table I. The majority of the metallic compounds tested were more effective in dilute solutions, both for a 90 and a 99.9 per cent kill, than in more concentrated solutions or in the solid state, probably because most of these chemicals have to be in intimate contact with the eggs or larvae in order to kill them. The majority of these compounds are, to some degree, larvicides. The metallic iodides and mercury compounds generally were the most potent, but most of the sulphates had low lethal values. The chlorides and nitrates had only moderate larvicidal activity. The same amounts of sodium, potassium, cupric, and calcium nitrates, applied as solids, were required to produce a 90 per cent kill, viz. 3.7 per cent. With the exception of ferrous and ferric chlorides, approximately twice the amount of solid metallic chlorides were required to produce a 99.9 per cent kill as were required for a 90 per cent kill.

Sodium compounds.—The most effective of the solid sodium compounds was sodium iodide; the next most effective was sodium fluoride. In solution sodium iodide was again the most effective, but sodium silicofluoride was comparable with sodium fluoride. Sodium tetraborate, both as a solid and in solutions, produced a 90 per cent kill. The low lethal value of sodium hydroxide was surprising, considering that it has been recommended as a disinfectant against hookworm larvae; however, it is one of the chemicals which sometimes makes third stage larvae exsheath and which sometimes kills them after they have developed. Sodium chloride, which has frequently been used against hookworm larvae, is only a moderately effective larvicide. Sodium bicarbonate and sodium hypochlorite both made some of the third stage larvae exsheath.

Solid sodium silicofluoride in practically all the cultures allowed some of the larvae to develop to the third stage before killing them; this characteristic was less marked when this chemical was added in solution. Sodium tetraborate applied as a solid in some cultures allowed the larvae to develop before they were killed, but only in a very few cultures did this occur when solutions of this chemical were applied. Solid sodium chlorate was four times as potent as solid potassium chlorate for a 90 per cent kill, and nearly six times for a 99.9 per cent kill.

Potassium compounds.—Of the solid potassium compounds, potassium iodide was the most lethal, and although it was about as effective as sodium iodide in producing a 90 per cent kill, it was more potent in producing a 99.9 per cent kill; in solutions these two compounds were comparable to each other. In the concentrations tested, potassium iodide and iodate were about equal and were the most lethal of the potassium compounds examined. Potassium iodate was 300 times as effective as potassium chlorate for 90 per cent and 56 times for 99.9 per cent kills; again indicating the possible effect of the iodine atom in the molecule.

Potassium chlorate appeared to be much less effective than sodium chlorate, and potassium hydroxide was slightly less effective than sodium hydroxide. Potassium hydroxide was like sodium hydroxide in that it sometimes made the larvae exsheath after the second ecdysis. Especially when applied as a solid, it sometimes killed the larvae after they had reached the third stage.

" Muriate of potash " (two grades) and potassium nitrate, both of which are used as fertilizers, gave similar results as larvicides. Kainite which was slightly more effective, and is also used as a potash fertilizer, is included in the magnesium compounds. Potassium sulphate, also a fertilizer, was rather less effective. Applied as a solid, potassium permanganate killed many of the larvae after they had reached the third stage and made some of the others exsheath, but it is a weak larvicide.

Ammonium compounds.—Included in the ammonium compounds are some nitrogenous fertilizers. All the ammonium compounds gave a 99.9 per cent kill when 10 per cent, or less, was added to the cultures. Ammonium iodide was the most effective, whether applied dry or in solution. It was found that to produce a 99.9 per cent kill ammonium sulphate, applied dry, was twice as lethal as sodium sulphate and three times more effective than potassium sulphate. When ammonium thiocyanate was applied either as a solid or in solutions in many of the cultures some of the larvae died after reaching the third stage. This happened in a few of the cultures treated with ammonium sulphamate, but fewer larvae died.

Copper compounds.—Of the four copper compounds tested copper sulphate, which is used as an anthelmintic, was the least effective. Cupric chloride was almost twice as lethal as the cuprous salt in producing 90 and 99.9 per cent reductions in the numbers of larvae. These copper compounds, however, killed many of the larvae only after they had developed to the infective stage (see Table IV); this occurred in the majority of the cultures to which these compounds were added in sufficient quantities.

Magnesium compounds.—Magnesium borate was the most effective of the few magnesium compounds tested; there was a tendency for this chemical to kill some of the larvae after they had developed.

Calcium compounds.—Most of the calcium compounds examined had little lethal value, although many farmers consider lime to be a larvicide, even on pastures. The heat of slaking is, of course, lethal, but otherwise only large quantities kill the larvae and then usually after they have developed.

As would be expected, calcium cyanide is very lethal, and was the most effective of the calcium compounds tested; there is sufficient moisture in faeces to make it release hydrogen cyanide. One per cent of calcium arsenate produced a 90 per cent reduction in the number of larvae, but 20 per cent was required to produce a 99.9 per cent reduction; however in most of the cultures treated with more than 1 per cent, only a few larvae developed and some of them died quickly. In some of the cultures treated with solid and very concentrated solutions of calcium chloroacetate some of the larvae died after they had reached the third stage.

Zinc compounds.—In common with the other metallic iodides which were tested, zinc iodide was the most effective of the zinc compounds, whether it was applied as a solid or in solution.

All four zinc compounds tested, including zinc iodide in very concentrated solutions, killed many of the larvae after they had reached the third stage. Zinc sulphide was very much less potent in reducing the numbers or in killing them after they had developed than zinc sulphate, which was one of the most effective sulphates.

Mercury compounds.—All the mercury compounds were lethal, especially ethyl-mercuric chloride. The larvicidal effect appeared to be depressed somewhat by the introduction of an ethoxyl group as was shown by the fact that ethyl-mercuric chloride was 15 times (for a 99.9 per cent kill) and 6 times (for a 90 per cent kill) as potent as ethoxyethyl-mercuric chloride, which had the same value as the unsubstituted mercuric chloride for a 90 per cent kill.

It is surprising that mercuric iodide was the least effective of the pure mercury compounds tested. This may be due to its insolubility. Levine (1951a) found that 0.011 per cent mercuric iodide produced a 99 per cent kill. We found that 2.5 per cent gave a 99 per cent reduction in the numbers of larvae, but that 1 per cent killed all the larvae which reached the infective stage, and considerably less killed most of them. In a few of the cultures treated with mercury compounds some of the larvae died after they had reached the third stage. In a few of the cultures treated with mercuric chloride, this chemical seemed to have had a selective lethal action as only a few of the larger strongyloid larvae were alive. Ethoxyethyl-mercuric chloride also showed some selective lethal activity.

Boron compounds.—Ortho-boric acid did not differ much in its lethal potency from sodium tetraborate and magnesium borate.

Carbon monoxide.—Sealing the jars containing the cultures is in itself lethal to developing larvae; it was therefore difficult to be sure that carbon monoxide remained in contact with the eggs and larvae for any length of time; however this gas did not appear to be lethal.

Sulphur.—There seemed to be a tendency for the numbers of larvae recovered from the cultures treated with sublimed sulphur to be greater than those from the controls. This can happen with a fungicide, which is not a larvicide, if there are fungi which parasitize larvae present in the cultures. In a few cultures to which nematocidal fungi were added the number of larvae was reduced by over 90 per cent in a few weeks.

Iodine.—Some iodine compounds have already been discussed; others will be in later sections (see Tables I and II). In the three samples which were tested, iodine in colloidal form lost some of its larvicidal potency.

Manganese compounds.—Neither manganous chloride nor manganous sulphate have a high larvicidal value. Applied in solid form the chloride was two or three times as effective as the sulphate; in solutions the differences were less. Manganous sulphate was one of the more effective sulphates. In a few of the cultures treated with both compounds some of the larvae died after they had developed to the third stage.

Iron compounds.—Ferrous iodide was the most potent of the iron compounds tested, but generally their larvicidal potency was low. Added to the cultures as solids, ferrous and ferric chlorides and ferrous and ferric sulphates were very similar in their effects. Some of the larvae in many of the cultures treated with the chlorides and sulphates of ferrous and ferric iron died after they had developed to the third stage, especially in the cultures treated with the ferrous compounds.

Cobalt and nickel compounds.—Cobalt and nickel chlorides were the only salts of these metals tested; they had somewhat similar lethal values. Nickel chloride had a greater tendency to kill the larvae after they had reached the third stage, but the numbers killed were never great, although quite small percentages killed a few.

Organic compounds (*aliphatic*)

The larvicidal values of the organic compounds are given in Table II.

Halogen derivatives of paraffins.—In this group are several chemicals which are used for the control of plant nematodes. Only a few of these compounds are appreciably soluble in water. Those which were tested in solution showed few significant differences from their values when applied undiluted. The most noteworthy feature of these derivatives is the lethal effect of the iodides (see Table III). The corresponding bromides were less lethal, but still had a considerable effect. The corresponding chlorides tested were found to be less effective than the bromides. Ethyl chloride (b.p. 12° C.) appeared to have a very low value, but it may have dispersed before some of the eggs had hatched.

Chloropicrin (nitrochloroform) affords an example of the apparent effect of a nitro-group. The substitution of a nitro-group for the hydrogen atom in chloroform seems to increase lethal activity 60 times for a 90 per cent kill and nearly 200 times for a 99.9 per cent kill. The amounts of chloropicrin required for 90 and 99.9 per cent kills were the same.

A comparison of carbon tetrachloride with carbon tetrabromide shows the latter compound to be nearly 70 times as effective for 90 per cent and almost 240 times for 99.9 per cent kills, again showing the influence of bromine in organic compounds. The results with carbon tetrabromide are sufficiently outstanding to justify further investigation into its nematocidal properties.

A comparison of the normal alkyl bromides is interesting. The percentage of chemical required to sterilize the faeces has been plotted against the number of carbon atoms in Fig. 1; the graphs for 90 and 99.9 per cent reductions are similar. The maximum quantity of chemical required was reached with *n*-amyl bromide followed by a sharp fall to *n*-hexyl bromide.

n-Butyl bromide was 12 times as powerful as *tert*.-butyl bromide for a 90 and 5 times for a 99.9 per cent kill.

A comparison of methylene dichloride, chloroform, and carbon tetrachloride shows that there is a decrease in potency with an increase in the number of chlorine atoms. In the corresponding bromo-compounds, the potency increases with the number of bromine atoms.

The iodine compounds are more effective than the corresponding bromine compounds, which, in turn, are more so than the corresponding chloro-derivatives (cf. alkyl halides).

Ethylidene dichloride is less effective than ethylene dichloride. DD, a mixture of 1 : 3-dichloropropylene, 1 : 2-dichloropropane and higher chlorides, had approximately the same larvicidal value whether applied undiluted or in the naphtha emulsion.

Iodoform was the only compound of this group which allowed some of the larvae to develop before they died; this happened whether the iodoform was mixed in the faeces or suspended above them; in the latter case larger quantities were required.

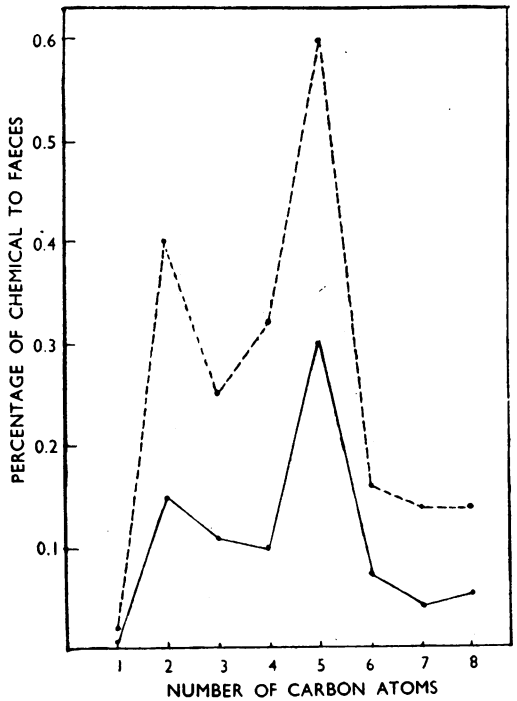

FIG. 1.—A graph to show the relationship between the number of carbon atoms in the *n*-alkyl bromides and the percentage of chemical required to produce a 90 per cent kill (continuous line) and a 99.9 per cent kill (broken line).

Halogen derivatives of ethylene.—An increase in the number of chlorine atoms results in increased potency in the chlorine derivatives of ethylene (cf. the chlorine derivatives of the paraffins). *Cis*- and *trans*-dichloroethylene were about equally effective in producing a 90 per cent kill, but the *cis*-isomer appeared to be slightly more powerful than the *trans*- for a 99.9 per cent kill. It may be that the proximity of the chlorine atoms to one another in the *cis*-compound is a contributory factor. The values for perchloroethylene were the same for 90 and 99.9 per cent kills.

Alcohols (methyl alcohol series).—It was found that effectiveness in producing a 90 per cent kill increased as the series of normal alcohols was ascended. *n*- and *iso*-propyl alcohols and *tert.*-butyl alcohol had the same value for a 90 per cent kill (1.5 per cent).

For a 99.9 per cent kill, methyl and ethyl alcohols were of equal effectiveness (5.0 per cent), and *n*-propyl, *n*- and *tert.*-butyl alcohols also had the same larvicidal values (1.5 per cent). The most interesting feature, however, was the greater larvicidal effect of *n*-propyl, *n*-butyl, and *tert.*-butyl alcohols, which were each twice as lethal as their *iso*-isomers. There were no significant differences whether these chemicals were applied undiluted or in aqueous solutions.

The addition of some compounds to the cultures occasionally produces third stage larvae which, although alive, are shrunken; a few of these larvae were found in cultures treated with methyl alcohol.

Ethers.—The two substituted diethyl ethers were more powerful larvicides than the unsubstituted compound. The substitution of two chlorine atoms for two hydrogen atoms increased the effect 30 times for a 90 per cent kill, and 36 times for a 99.9 per cent kill. In some cultures treated with dichlorodiethyl ether a few of the larvae died after they reached the third stage.

Sulphides.—Tetramethylthiuram monosulphide and the corresponding disulphide, both of which were applied in slight suspension, were very much more effective when added to the cultures in this way than when they were added dry. In most suspensions the disulphide, although 54 per cent of the mixture added was an inert dispersal agent, was more effective than the monosulphide in reducing the numbers of the larvae; but in the cultures treated with the monosulphide there was a greater tendency for many of the larvae to die after they had reached the third stage.

Formaldehyde derivative.—A few of the larvae in cultures to which hexamethylene tetramine had been added died after they had reached the third stage.

Esters.—Ethyl iodoacetate, like other lachrymators, has a fairly high larvicidal value. With the exception of this compound, the other esters which were tested all allowed some larvae to reach the third stage before killing them; this characteristic was very marked when hexaethyl tetraphosphate, diethyl-*p*-nitrophenylthiophosphate or Teepol X were added undiluted.

Amines.—In many of the cultures to which dimethylamine had been added as a 25 per cent aqueous solution dead eggs were found, and a few were found in four cultures to which weaker solutions had been added. When this compound was added to the cultures in small quantities some of the larvae reached the third stage before they were killed.

Cetyltrimethylammonium bromide, a wetting agent, and the 20 per cent solution of alkylammonium bromides, dissolved in methylated spirit, both killed some larvae slowly, so that they had reached the third stage before they died, but this occurred much more frequently with the former.

Allyl compounds.—The allyl compounds tested were very effective larvicides, particularly allyl isothiocyanate (a lachrymatory liquid) and allyl iodide, which were approximately equal. Allyl isothiocyanate and allyl alcohol, the two allyl compounds which were tested as aqueous solutions, showed very little difference in value whether applied undiluted or in solutions. When the allyl halides were compared, it was found, as with the alkyl halides, that allyl iodide was more powerful than the bromide, which in turn was more effective than the chloride. Compared with alcohols of the methyl alcohol series, allyl alcohol (a lachrymatory liquid) was much more potent.

Amino acid.—The addition of cystine to the cultures even in large proportions only reduced the number of larvae slightly, but a few more died after they had reached the third stage.

Carbonic acid derivatives.—Both zinc and ferric dimethyldithiocarbamates were much more effective applied in suspension than added dry. Small quantities of both compounds markedly reduced the numbers of larvae which reached the third stage and quite small quantities killed some of the larvae after they had developed; however, not even large quantities of the dry compounds prevented all the larvae developing to the third stage or killed all that did so.

The substitution of a sulphur atom for oxygen in urea increased the effect eight times for 90 per cent and twice for 99.9 per cent reductions in the number of larvae, when the compounds were applied as solids. In solution, thiourea was generally more effective, and, in addition, many of the larvae in cultures treated with thiourea solutions died after they had developed to the third stage.

The frequency with which some larvae developed to the third stage and then died was marked in cultures treated with methylguanidine sulphate, diphenyl guanidine, and creatinine in the more concentrated solutions; this also occurred in the more concentrated solutions of guanidine hydrochloride, *as*-dimethylguanidine sulphate and creatine, but to a less marked extent.

Ureides and purine derivatives.—Although the addition of 50 per cent of allantoin in the dry state to the cultures did not reduce the number of third stage larvae found by more than about 99.7 per cent, the addition of only 1.25 per cent ensured that they had all died after they had developed to the third stage.

Both xanthine and guanine added to the cultures of fresh horse faeces as solids killed some of the third stage larvae.

Organic compounds (carbocyclic)

Hydrocarbons.—The xylenes (*ortho-*, *meta*, and *para*-mixed) and *p*-cymene were equally potent and very effective larvicides, more so than toluene, which was more effective than benzene. It would appear that an increase in the number of alkyl groups increases the larvicidal effect.

Halogen derivatives.—Chloro-, bromo-, and iodo-benzene, *o*- and *p*-dichloro-benzene, and cyclohexyl bromide were quite effective. Benzene hexachloride (containing a mixture of isomers) and its α-, β-, γ-, and δ-isomers separately, and DDT were all tested as solids, but had low or no larvicidal values. However, in many of the cultures to which 3.75 per cent or more of benzene hexachloride was added some of the larvae died after reaching the third stage, but even 50 per cent of benzene hexachloride only reduced the number of larvae which were extracted from the cultures by about 80 per cent.

Chloro-, bromo-, and iodo-benzenes were tested in very dilute solutions, but they did not affect the eggs or larvae.

Although the addition of 50 per cent of 2:3:5:6-tetrachloronitrobenzene to fresh horse faeces did not quite reduce the number of larvae recovered by 99.9 per cent, yet the addition of only 1.9 per cent ensured that some of the larvae died after reaching the third stage.

Mercury compounds.—The two mercuric compounds confirm the lethal effect of the mercury atom, which was noticed in the inorganic and aliphatic compounds of mercury. Both tended to kill some of the larvae after they had developed.

Amino-compounds.—Aniline is an effective larvicide, but the other compounds, which were tested, were not so effective in reducing the numbers of the larvae. However, diphenylamine in small quantities affected the viability of the larvae; for example, in some cultures treated with 0.5 per cent, and over, a considerable number of larvae were alive when they were extracted from the cultures, but ten days later all or practically all, had died. Diphenylamine tended to darken the larvae.

Azo-compounds.—In cultures treated with azobenzene and with aminoazobenzene hydrochloride some larvae died after they had reached the third stage. Aminoazobenzene hydrochloride makes the larvae sluggish; therefore it is probable that a lower proportion of live larvae than normal was recovered from the cultures. This compound occasionally stains live larvae.

Phenols.—The relative effectiveness of the phenols was increased by dilution with water. The low boiling-point phenols were more effective than the less volatile phenols.

Filix mas emulsion and 4-*n*-hexylresorcinol, two compounds with specific anthelmintic properties, have low larvicidal potencies. Apart from chemical reasons this apparent anomaly may be due to the physical condition of the Filix mas emulsion and to the poor solubility of 4-*n*-hexylresorcinol (1 : 2,000) in water, which would only allow it to penetrate the faeces slowly. There is, perhaps, confirmation of this in the fact that many larvae in cultures treated with 4-*n*-hexylresorcinol develop to the third stage before being killed. Resorcinol, on the other hand, is quite effective; so are phenol, sodium 2 : 4 : 5-trichlorophenate, *o*-nitro-phenol, and thymol.

The same amount of *o*-nitro-phenol is required for a 90 as for a 99.9 per cent kill. This compound is 5 times as effective for a 90 per cent and 20 times as effective for a 99.9 per cent kill as the *para*-isomer. Sodium 2 : 4 : 5-trichlorophenate has about the same effect as phenol, but the larvicidal effect is considerably reduced in sodium *o*-phenylphenate and still more so in sodium *m*-chloro-*p*-phenyl phenate. The introduction of a phenyl group may be the contributory factor.

Among the phenols were compounds which appeared to kill the eggs and many which allowed some of the larvae in the cultures to develop before killing them. These apparent contradictions may be due to the very local action of some of these compounds. For example, when the chemicals were mixed in the cultures they would immediately be in contact with some eggs; during development, more larvae would come in contact with the chemicals, but some might not do so until they had developed to the third stage.

Ketone.—*p*-Chloroacetophenone is another lachrymatory compound, but its comparatively low vapour pressure may account for the fact that its larvicidal potency is lower than that of the other lachrymators, and for the fact that some larvae in the cultures were able to develop to the third stage before being killed.

Monobasic acid.—Bloomfield (1949) found that benzoic acid was the only constituent of urine which killed eggs, preinfective and infective larvae. In the present investigation, the addition of 50 per cent of benzoic acid only reduced the number of larvae by 97 per cent; 30 per cent was the minimum percentage which killed all the larvae which reached the third stage in all the cultures although in some cultures all the larvae were killed by smaller quantities. This suggests that this compound must be in close contact to be lethal to eggs or larvae.

Esters of dibasic acids.—Dimethylphthalate had a very low lethal value, but it was more potent than the dibutyl ester, and killed many of the larvae which developed to the third stage, which dibutylphthalate did not do.

Triphenylmethane dyes.—Added as solids these dyes have a low potency as larvicides, although some of them are recognized anthelmintics against some nematodes which parasitize man. Gentian violet was much more effective in solution than in the solid state in producing a 90 per cent kill, but did not produce a 99.9 per cent kill. In some of the cultures treated with these dyes some of the third stage larvae, both live and dead, were stained; the staining was particularly frequent in the larvae from cultures treated with magenta, methyl violet, and gentian violet. As some of the larvae died fairly soon after reaching the third stage (see Table IV) it seems probable that these dyes have a slow lethal action on the free-living stages in faeces at 25° C.

Terpenes.—Turpentine, essential oil of *Artemisia maritima*, containing 65 per cent β-thujone and 16 per cent 1 : 8-cineole, and oil of chenopodium were effective larvicides, but santonin was of no value. This may be caused by its physical condition, although as a 1 : 500 aqueous solution 25 c.c. in 40 g. of fresh faeces only caused a 70 per cent reduction in the number of third stage larvae which were found; it is really a vermifuge.

1 : 8-Cineole and β-thujone were tested separately and found to be effective. β-Thujone had the same value for 90 and 99.9 per cent kills, and was 6 times more potent than 1 : 8-cineole for a 99.9 per cent kill.

Naphthalene and β-naphthol.—Judged by the numbers of larvae which developed to the third stage, the larvicidal effect of naphthalene was weakened very considerably by the introduction of the hydroxyl group in the β-position. This was rather unexpected as the introduction of a hydroxyl group sometimes increases anthelmintic activity. Many of the third stage larvae died in the cultures treated with β-naphthol, but even for this delayed killing effect considerably more β-naphthol was required than was necessary to produce a 99.9 per cent reduction in the numbers of larvae in the naphthalene treated cultures.

Organic compounds (heterocyclic)

Benzthiazole.—In practically all of the cultures treated with 2.5 per cent, or more, of 2-mercapto-benzthiazole some of the larvae which reached the third stage rapidly died, although 35 per cent was required to produce even a 90 per cent reduction in the number of larvae recovered from the cultures.

Pyridine derivatives.—Pyridine and derivatives were found to be effective larvicides and had values ranging from 0.09 to 0.23 per cent for 90 per cent and from 0.17 to 0.25 per cent for 99.9 per cent kills. The lethal values of these chemicals were little changed on dilution with water.

Quinoline and derivative.—In many of the cultures treated with undiluted quinoline and with concentrated solutions of 8-hydroxyquinoline sulphate many larvae died after they had reached the third stage; some larvae died in a few of the cultures treated with these two compounds in less concentrated solutions.

Acriflavine.—Acriflavine sometimes killed the larvae after they had reached the third stage, yet at other times the third stage larvae were deeply stained but were apparently unharmed.

Benzthiazine derivatives.—These derivatives had very little larvicidal value; the simplest compound, 3-keto-2 : 3-dihydrobenz-1 : 4-thiazine, was the most lethal. In the cultures treated with the aminohydrochloride many of the larvae died after they had reached the third stage; this compound adhered to their sheaths.

Phenoxthine.—Although the addition of very small quantities of phenoxthine to cultures reduced the numbers of larvae which reached the third stage very considerably, and many of the larvae which did reach that stage died, yet even 50 per cent of phenoxthine added to the cultures did not prevent some larvae developing. In twenty out of forty-five cultures treated with 2.5 per cent, or more, of this compound all the larvae were dead.

Phenothiazine and derivatives.—Mercurated phenothiazine was a powerful larvicide, but phenothiazine and the other phenothiazine derivatives applied as solids were not effective larvicides. Phenothiazine, phenothiazone, and methylene blue were more effective in producing a 90 per cent kill in solution or suspension than when applied dry, but none of these chemicals produced a 99.9 per cent kill. The fact that in each culture of horse faeces there are larvae of many species probably decreases the chance of a 99.9 per cent kill with any chemical which is a specific larvicide; as an anthelmintic phenothiazine is selective.

Lauth's violet was not particularly effective, but when coupled with phenol, its potency was slightly increased. Other couplings with Lauth's violet decreased its value; but many of the larvae died after they reached the third stage in cultures treated with Lauth's violet$\rightrightarrows$(β-naphthol)$_2$, some in the cultures treated with Lauth's violet$\rightrightarrows$(2-hydroxy-3-naphthoic acid)$_2$, with phenothiazone and with over 30 per cent of methylene blue.

Alkaloids.—Of the alkaloids added as solids to the cultures arecoline hydrobromide was the most effective. Nicotine sulphate (40 per cent solution) diluted twenty to fifty times with water was considerably more effective than in the more concentrated solution.

Plant products.—The plant products which were tested had very little larvicidal value. The crushed leaves were tried in order to determine whether or not they might slowly release sufficient hydrogen cyanide to be of value in destroying plant nematodes, if crops with high nitrogen contents were ploughed in as green manure.

Urines and manure.—Although fresh poultry manure and urines have a low larvicidal value, it has been found that they have a practical value for killing larvae in manure heaps and in night-soil.

Physical properties

In general there appeared to be no correlation between boiling-points and larvicidal effect. In the inorganic compounds there seemed to be a general tendency for larvicidal effect to increase with solubility in water, but there were exceptions. In the organic compounds no increase in larvicidal effect was observed with increase of solubility, when the chemicals are taken as a whole. It would appear that, unless the chemical has to be in intimate contact with the eggs or larvae, solubility in water is of secondary importance. No simple correlation exists between larvicidal action and molecular weight, vapour pressure or parachor.

Conclusions

Many chemicals were applied to fresh horse faeces in a confined space at a temperature of about 25° C.; from the results it has been possible to draw some conclusions on their effect as larvicides against the free-living stages of Sclerostomes.

1. Of the metallic compounds which were tested, those of mercury were the most potent larvicides.
2. The metallic iodides are generally the most potent of the inorganic salts; compounds of fluorine and boron are less effective larvicides.
3. The nitrates have comparable larvicidal values to the chlorides, both of which are more potent than the sulphates.
4. The ammonium compounds are larvicidal and have a practical value in killing larvae in night-soil and middens.
5. The copper compounds are notable for the slowness with which they kill Sclerostome larvae.
6. Calcium compounds, in spite of their reputation, are very weak larvicides.
7. Sulphur has no larvicidal action.
8. Most inorganic compounds are more effective when applied in solution than as solids.
9. Iodo-derivatives of the paraffins are very lethal, the order of potency of halogenated paraffins being iodo>bromo>chloro.
10. The substitution of a nitro-group for the hydrogen atom in chloroform considerably increases its larvicidal value.
11. The larvicidal value of carbon tetrabromide is high; this chemical should be tested against other helminths.
12. *n*-Amyl bromide is the least effective of the normal alkyl bromides in the series methyl to octyl.

13. *n*-Butyl bromide is more lethal than *tert.*-butyl bromide.

14. In the halogen derivatives of the paraffins there is a decrease in larvicidal potency with an increase in the number of chlorine atoms, but an increase in potency with an increase in the number of bromine atoms.

15. In the halogen derivatives of ethylene an increase in the number of chlorine atoms increases the larvicidal potency.

16. The effectiveness of the normal alcohols to produce a 90 per cent reduction in the number of larvae increases as the series of alcohols is ascended.

17. *n*-Propyl, *n*-butyl, and *tert.*-butyl alcohols are more lethal than their *iso*-isomers.

18. The substitution of two chlorine atoms for two hydrogen atoms in ether considerably increased the larvicidal value.

19. Tetramethylthiuram disulphide is more effective than the monosulphide in reducing the number of larvae, but both have to be in intimate contact with the larvae to kill them.

20. Dimethylamine and diphenylamine are both ovicidal as well as larvicidal, but the latter has a peculiar delayed larvicidal action.

21. Allyl compounds are very potent larvicides, especially the isothiocyanate and the iodide. With the halides, the order of potency is I>Br>Cl.

22. Both zinc and ferric dimethyldithiocarbamates have to be in intimate contact with larvae to kill them.

23. The substitution of a sulphur atom for oxygen in urea increases its larvicidal value considerably.

24. Many of the carbonic acid derivatives have a slow lethal action.

25. In the aromatic hydrocarbons an increase in the alkyl groups increases the larvicidal values.

26. Chloro, bromo- and iodobenzene, *o*- and *p*-dichlorobenzene are quite potent larvicides, but benzene hexachloride has a very low larvicidal value, and 2: 3: 5: 6-tetrachloronitrobenzene is a very slow killer.

27. Azobenzene and aminoazobenzene hydrochloride also seem to have a delayed lethal effect.

28. A number of phenolic compounds are effective larvicides, but several of them, including sodium 2: 4: 5-trichlorophenate, sodium-*o*-phenyl phenate, and sodium *m*-chloro-*p*-phenyl phenate, allow many of the larvae to develop to the third stage before they are killed. The introduction of a phenyl group reduces the larvicidal value of sodium 2: 4: 5-trichlorophenate. *o*-Nitro-phenol is much more effective than the *para*-isomer.

29. Some of the triphenylmethane dyestuffs also have a delayed lethal action.

30. The value of β-thujone should be tested against other helminths.

31. β-Naphthol is a less effective larvicide than naphthalene.

32. Pyridine and its derivatives are effective whether applied undiluted or as solutions.

33. As larvicides the benzthiazine derivatives have low values.

34. Phenoxthine has a slow lethal effect.

35. Phenothiazine and its derivatives, except mercurated phenothiazine, have low larvicidal values, but were more effective in solutions.

36. Urines and poultry manure, because of their availability on farms and their value as fertilizers, can be used in well-made middens to kill bursate nematode larvae, although their larvicidal potency is low.

37. The lachrymators, which were tested, have high larvicidal values.

38. Solubility is no guide to larvicidal effect; except in a number of inorganic compounds, where larvicidal effect increases with increase of solubility in water.

39. There is no correspondence between boiling-points, molecular weights, vapour pressures and parachors, and larvicidal values.

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REFERENCES

Baldwin, E. (1943). *Parasitology*, **35**, 89.
Baldwin, E. (1948). *Brit. J. Pharmacol.*, **3**, 91.
Bloomfield, S. S. (1949). *Canad. J. comp. Med.*, **13**, 277.
Chance, M. R. A., and Mansour, T. E. (1949). *Brit. J. Pharmacol.*, **4**, 7.
Duguid, A. M. E., and Heathcote, R. St. A. (1950). *Arch. int. Pharmacodyn.*, **82**, 309; **84**, 159.
Lamson, P. D., and Brown, H. W. (1936). *Amer. J. Hyg.*, **23**, 85.
Levine, N. D. (1949). *Amer. J. vet. Res.*, **10**, 233.
Levine, N. D. (1951a). *Amer. J. vet. Res.*, **12**, 110.
Levine, N. D. (1951b). *J. Parasitology*, **37**, 195.
Parnell, I. W. (1936). *Canad. J. Res. D.*, **14**, 71.
Parnell, I. W. (1937). *Canad. J. Res. D.*, **15**, 127.
Parnell, I. W. (1938). *Canad. J. Res. D.*, **16**, 73.
Parnell, I. W. (1940). *Canad. J. Res. D.*, **18**, 371.
Wright, W. H., and Schaffer, J. M. (1932). *Amer. J. Hyg.*, **16**, 325.