THE TOXICITY OF THE DINITRO-CRESOLS

PART I. 4:6 DINITRO-*ortho*-CRESOL AND ITS SIMPLER DERIVATIVES

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INTRODUCTION

4:6-Dinitro-*o*-cresol* is widely used as an ovicide in winter washes for fruit trees and in the spring and early summer for the control of weeds in corn (David[1]). A recent report also indicates that it may prove to be effective as an anti-locust measure.[2] Attention has been drawn to the dangers likely to be encountered in its use (Bidstrup and Payne[3]) and to the fact that it acts as a cumulative poison in man (Harvey, Bidstrup and Bonnell[4]; Bidstrup, Bonnell and Harvey[5]). Suggestions have been made that its toxicity may be complicated by the presence of isomeric dinitro-cresols that may be formed during the course of its manufacture. This theory has also been advanced by Molnar[6] who has described extensive toxicological tests on two isomeric dinitro-*o*-cresols. Although one of these has properties that suggests that it is probably identical with 4:6-dinitro-*o*-cresol Molnar has neither specified the structure nor detailed the manufacturing processes of either compound. A discussion of this aspect will be included in a later communication.

The present paper deals with a general survey of the physical properties and toxicity of some commercial samples of 4:6-dinitro-*o*-cresol and their simpler derivatives which have been manufactured in the United Kingdom.

MANUFACTURE

A process at present employed is essentially that described by Nolting and Salis.[8] High grade *o*-cresol is sulphonated with sufficient sulphuric acid to give the 4:6-disulphonate derivative. The mixture containing this substance is then treated with nitric acid at a temperature above the melting point of the nitro-compound. This ensures complete nitration. The crude nitro-compound is separated from the acid mixture while still in the molten state. This reduces the amount of occluded impurities. The crude product is washed with cold water to remove the last traces of mineral acids. It is then packed in drums while still in the moist state. This is then ready for despatch. Most solutions are made up as ammonium or sodium salts since these are more soluble than dinitro-*o*-cresol itself.

PHYSICAL PROPERTIES OF SOME COMMERCIAL SAMPLES

The moisture content, solubility at 18° C., melting points and mixed melting points with pure samples were determined in the usual way. 7

* An alternative name is 3:5-dinitro-*o*-cresol. This is used by Parker, Barnes and Denz.[7]

TABLE I

PHYSICAL PROPERTIES OF SOME COMMERCIAL SAMPLES OF 4:6-DINITRO-*o*-CRESOL AND ITS DERIVATIVES

Sample	Mol. wt.	Descriptions index letter	Colour	Moisture per cent.	Solubility H_2O(18°C.)	Melting point °C. (*a*)	Mixed melting point °C.	Recovery of dinitro-*o*-cresol in 1 per cent. solution Theory 1 per cent. (*b*)
4:6-Dinitro-*o*-cresol	198	Pure recryst.	Bright yellow	nil	0·024	86·0	—	0·96
		A	Pale brown	15·0	0·018	86 to 88	86 to 86·5	0·99
		B	Yellow brown	10·7	0·022	84 to 86	82 to 86	0·88
		C	Yellow	0·07	0·017	86 to 88	85 to 89	1·00
		D	Pale brown	6·0	0·021	83 to 86	84 to 86	1·00
		E	Brown	4·0	0·021	84 to 87	83 to 89	0·99
		H	Pale brown	7·9	0·017	84 to 88	85 to 89	0·99
		K	Yellow	9·9	0·018	84 to 87	82 to 89	1·00
Ammonium	214 (+8·5)	Pure recryst.	Bright yellow	nil	0·91	Decomp.	—	0·88 (−8·3) (*c*)
Salt		G	Bright yellow brown	1·3	0·98	Decomp.	Decomp.	0·88
Diethylamine	272 (+37)	Pure recryst.	Yellow	nil	7·4	110 to 111	—	0·73 (−25) (*c*)
Salt		F	Yellow	4·7	7·5	108 to 110	93 to 106	0·69 (−17) (*c*)
Sodium salt	220 (+11)	J	Golden yellow	21·4	7·9	Decomp.	—	0·79 (−17) (*c*)
4:6-Dinitro-*sec.*-butylphenol (*d*)	240 (+21)	95 per cent. pure	Yellow	nil	0·004	45	45	0·75 (−22) (*c*)
		Pure recryst.	Yellow	nil	0·006	45	—	0·80

NOTES.

(*a*) Melting points uncorrected. Material dried *in vácuo* over sulphuric acid before use. Heilbron and Bunbury[9] give 86·5° as m.pt. of pure 4:6-dinitro-*o*-cresol.

(*b*) 1·00 g. dissolved in slight excess of aqueous sodium bicarbonate and the solution made up to 100 ml.

(*c*) The reduction in the amount of dinitro-*o*-cresol estimated by Parker's method[10] is approximately equivalent to the increase in the molecular weights of the salts and consequent diminution of "active" compound per unit volume of solution.

(*d*) 4-6-Dinitro-*sec.*-butylphenol has similar chemical and physiological properties to 4:6-dinitro-*o*-cresol. It can also be estimated by the method of Parker (*loc cit.*)

commercial samples of dinitro-*o*-cresol and 1 each of the sodium, ammonium and diethylamine salts were thus examined. In addition a sample of 4:6-dinitro-*sec.*-butyl phenol was included in the survey since this substance is also used in spray operations, although not to the same extent as dinitro-*o*-cresol. Table I lists the results of the examinations.

In view of the possibility of the occurrence of additional isomers or toxic impurities an attempt was made to remove them on an alumina

column. With the exception of a small quantity of dark brown amorphous benzene insoluble material no substances other than 4:6-dinitro-*o*-cresol could be isolated from the samples examined. Relative purity of the substances examined was also expected from the small depressions obtained from the mixed melting points.

Toxicity Tests

All solutions of the samples were made up to 1 per cent. w/v by dissolving exactly 1·0 g. of the material (dried at 60° C. and then over sulphuric acid *in vacuo*) in 1·0 per cent. saline solution containing 1·0 per cent. of sodium bicarbonate and making up to 100 ml. The solutions were administered by subcutaneous injection. Pure recrystallised 4:6-dinitro-*o*-cresol was made up as a 1 per cent. solution in a similar manner and used as a reference throughout. Pilot experiments were carried out to determine the approximate LD50 on small groups of about 4 rats per group. Thereafter 10 animals were used per dose level. 2 or 3 dose levels were selected, the mortalities over 24 hours converted into probit values which were plotted against log doses, and the LD50 calculated in the usual way.

In view of the importance of the alimentary canal as route of absorption of dinitro-*o*-cresol and the possible effect of heat in stimulating its metabolic effects (Bidstrup and Payne, *loc. cit.*, Barnes *et al. loc. cit.*, King and Harvey[11]) a further simple comparative test was carried out. This consisted of administering by stomach tube a single sub-lethal dose to rats and noting their response and mortality to 4 hours exposure at 20° to 22° C., 5 hours at 37° to 40° C. and 15 hours at 20° to 22° C. The dose level was 50 mg./kg., and the samples were dissolved in aqueous bicarbonate solution to give 1 per cent. strength. 10 rats were used per sample. The results of both toxicity tests are given in Table II.

Discussion

The evidence presented indicates that the commercial samples that have been examined are slightly less toxic than the pure compound and that the ammonium, sodium and diethylamine salts are also less toxic than dinitro-*o*-cresol. Also that 4:6-dinitro-*sec.*-butylphenol is slightly more toxic than dinitro-*o*-cresol. Indirectly this also suggests that if the manufacturing process is carried out in general agreement with the method of Nolting and Salis, using high grade *o*-cresol uncontaminated by the *meta* and *para* isomers there will be little likelihood of any other dinitro-cresols or by-products being formed in any quantity.

The two values obtained for the LD50 on the two series of rats are in fairly close agreement with the values quoted by Ambrose,[12] 25 mg./kg., and by Parker *et al.* (*loc. cit.*), 24·6 mg./kg.

Although the diethylamine salt is nearly half as toxic as pure dinitro-*o*-cresol this can probably be correlated with the increased molecular weight and the consequent reduction of the amount of "active" compound per unit volume of its solution in water or other solvent. It is possible that the decreased toxicity and the increased solubility of this salt may have

some practical bearing on its use in the field. In the first place comparison of the "effective" quantities of the diethylamine salt and other derivatives required for weed killing suggests that less of the former compound may be required.

The second point is that the increased solubility of the diethylamine salt aids its dispersion since it does not sediment readily in the storage tanks and will not block the nozzles in the spray beam. This means that the

TABLE II

TOXICITY OF SOME COMMERCIAL SAMPLES OF 4:6-DINITRO-*o*-CRESOL AND THEIR DERIVATIVES

Substance	Subcutaneous injection LD50		Stomach tube, mortality out of 10			
	1st series (albino rats)	2nd series (hooded rats)	4 hours 20° to 22° C.	5 hours 37° to 40° C.	15 hours 20° to 22° C.	Total per cent.
4:6-Dinitro-*o*-cresol pure ..	25·6	28·5	0	7	0	70
,, Comm. A	26·2	—	3	7	0	100
,, B	26·8	—	3	7	0	100
,, C	27·5	—	2	8	0	100
,, D	—	—	0	9	0	90
,, E	—	—	0	10	0	100
,, H	26·8	—	0	9	0	90
,, K	—	—	1	7	0	80
Ammonium salt, pure	—	—	0	6	1	70
Comm. G ..	—	27·5, 30·0	0	10	0	100
Diethylamine salt, pure	—	36·5, 39·1	0	1	2	30
Comm. F	—	—	0	8	0	80
Sodium salt (*a*) ..	—	—	0	9	0	90
Comm. J, 4:6-dinitro-*sec*-butyl phenol, 95 per cent.	—	21·4	0	6	4	100

NOTE.—(*a*) This was not assayed as all other samples of 4:6-dinitro-*o*-cresol were administered as solutions of their sodium salts.

spray operator will not need to clear the nozzles and therefore will not incur the risk of a sudden shower of spray (cf. Bidstrup and Payne[3]). In spite of these practical points, which are of obvious importance, it must be remembered that increased solubility may increase absorption and every effort must be made to continue protective devices.

From this study it can be concluded that all the dinitro-*o*-cresol compounds which have been investigated are dangerous. Although the diethylamine salt is less toxic than the other compounds studied it appears to have similar physiological effects at normal and elevated environmental temperatures. The lower toxicity of the diethylamine salt suggests a profitable line of research in finding other derivatives of dinitro-*o*-cresol which, even if they have the same qualitative effect may be quantitatively less active as human poisons.

SUMMARY

1. Commercial samples of dinitro-*o*-cresol and their common salts have been examined by physical and toxicological tests.

2. Commercial samples of dinitro-*o*-cresol and its salts are less toxic than the pure substance.

3. The diethylamine salt is about 50 per cent. less toxic than pure 4:6-dinitro-*o*-cresol.

4. 4:6-Dinitro-*sec.*-butylphenol is slightly more toxic than 4:6-dinitro-*o*-cresol.

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