RESEARCH PAPERS

THE TOXICITY OF THE DINITRO-CRESOLS

PART II. THE FORMATION AND TOXIC PROPERTIES OF SOME NITRO-COMPOUNDS DERIVED FROM *meta*- AND *para*-CRESOLS

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INTRODUCTION

IN Part I of this series (Harvey[1]) it was shown that some commercial samples of 4:6-dinitro-*o*-cresol* (DNOC) were relatively pure and that their LD50 values for rats were similar to that of the pure substance. This observation appeared to exclude the possibility that significant quantities of other nitro-cresols might be formed in side reactions during the manufacture of dinitro-*o*-cresol.

Molnar's[2] report of two unidentified dinitro-cresols has stimulated further research on the possible identity of these compounds. This has led to a fuller investigation of the original reaction of Nolting and Salis[3] for the preparation of dinitro-*o*-cresol from *o*-cresol. In addition, this reaction has been applied to *m*- and *p*-cresols, both of which might occur as "natural" contaminants of *o*-cresol.

The toxicities of the main products of the various reactions have also been studied as single substances and as mixtures.

The nitration of o-, m- and p-cresols

Heilbronn and Bunbury[4] and Beilstein[5] list respectively 10 and 8 known dinitro-cresols out of a theoretical 18 isomers. Of these only 2 are prepared by direct methods of nitration. They are the well known 4:6-dinitro-*o*-cresol and 2:6-dinitro-*p*-cresol (DNPC), also known as Victoria Yellow or Victoria Orange, and originally incorporated in a mixture containing the ammonium salts of the two compounds. This was used as a colouring agent for foodstuffs until its poisonous nature was discovered.

The first stage in the preparation of dinitro-*o*-cresol is the sulphonation of the *o*-cresol. The structure of the resulting product depends on several factors. These include the temperature at which the reaction is conducted and the quantity of sulphuric acid used. 4:6-Disulphonic-*o*-cresol is the main product if excess of sulphuric acid is employed and if the reaction is carried out in a temperature range of 80° to 100° C. Limited quantities of sulphuric acid and variations of temperature result in at least three *o*-cresol monosulphonates (Datta and Mitter[6], Claus and Jackson[7],

* The descriptions of all chemical substances described in this paper have been made to conform with the terminology that describes DNOC as 4:6-dinitro-*o*-cresol and *not* 3:5-dinitro-*o*-cresol.

Engelhardt and Latschinow[8]). Subsequent treatment of the disulphonate by nitric acid (Nolting and Salis, *loc. cit.*) or by nitrous fumes (Datta and Varma[9]) leads to the formation of dinitro-*o*-cresol in good yield and purity. According to Datta and Varma, *loc. cit.*, nitration of the 5- or 6-monosulphonates by nitrous fumes leads to the formation of 5:6-dinitro-*o*-cresol. Claus and Jackson, *loc. cit.*, and Nevile and Winther[10] claim that concentrated nitric acid reacts with the 4- and 6-monosulphonates to yield dinitro-*o*-cresol.

Sulphonation and nitration of *m*-cresol yields 2:4:6-trinitro-*m*-cresol[11] (TNMC). This compound is also known as cresylite, an explosive with similar properties to picric acid.

2:6-Dinitro-*p*-cresol is readily formed by several simple methods of nitration. These include treatment of *p*-cresol by nitric acid in acetic acid (Frische[12]) by nitric and sulphuric acids (Borsche and Fiedler[13]) and the action of nitric acid on 2:6-disulphonic-*p*-cresol (Nolting and Kohn[14]). Datta and Varma, *loc. cit.*, have prepared dinitro-*p*-cresol by treating the sulphonate with nitrous fumes.

Other methods can be used for the preparation of dinitro-*o*-cresol, trinitro-*m*-cresol and dinitro-*p*-cresol, but they are of academic rather than practical interest. They include the treatment of the cresotinic acids (Datta and Varma, *loc. cit.*) and of the toluidines (Datta and Varma[15]) by nitrous gases. The main reactions and products involved in the "full" nitration of the cresols are summarised in Figure 1. The reactions and products specially studied in this communication are printed in thick type.

Experimental

As the main object of the experiments was to apply the Nolting and Salis reaction to the three cresols and to isolate and identify the main products no attempt was made to examine each reaction exhaustively, or to isolate any very small quantities of possible by-products. The method employed for nitrating the cresols was essentially as follows.

A mixture of the cresol (1 mol.) and concentrated sulphuric acid (A.R. approx. 2·2 mols.) was heated on a boiling water bath for 2 hours. The resulting mixture was poured into water to give a 5- to 10-fold dilution and treated with nitric acid (A.R. approx. 2·2 mols., sp.gr. 1·42) in small additions with good shaking or stirring. As all the reactions studied were variably exothermic the temperature was raised to 100° to 105° C. in order to obtain uniformity of heat conditions in all preparations. After cooling, the separated solid was filtered off, well washed with cold water, suspended in hot water, again cooled, filtered, and finally recrystallised from ethanol (96 per cent.). Melting points were determined by capillary tube method and were uncorrected. Microanalyses for carbon, hydrogen and nitrogen were carried out by Drs. Weiler and Strauss of Oxford.

Fractional crystallisation of suspected mixtures proved to be laborious and unsatisfactory, therefore chromatographic separation on active alumina was employed. Ultra-violet absorption spectra data and curves

of the chief compounds were obtained by Dr. E. R. Holiday of the Spectrographic Unit, Medical Research Council. Visible absorption spectra curves were made on a Unicam spectrophotometer.

LD50 values on the products were obtained for mice in a manner similar to that described by Harvey, *loc. cit.*, for testing commercial dinitro-*o*-cresol samples. Blood decay rates were determined by administering single 20 mg./kg. doses of solutions of the substances

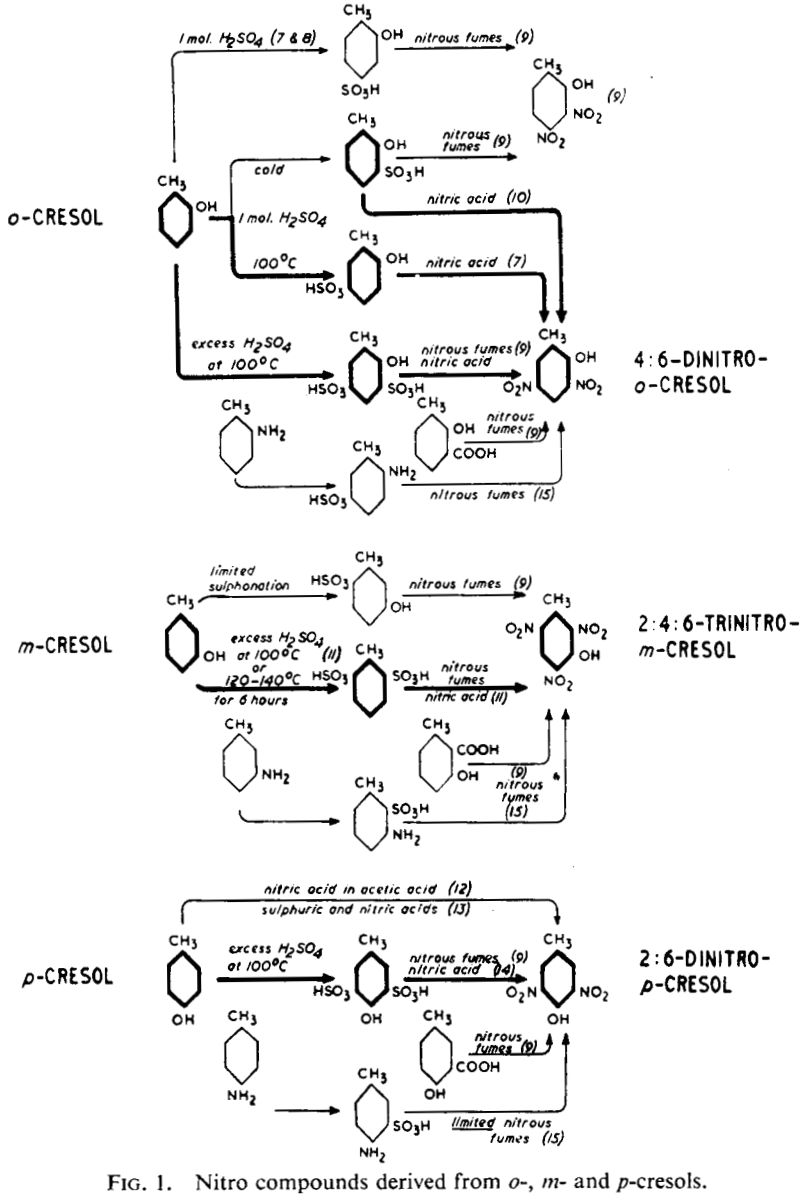

FIG. 1. Nitro compounds derived from *o*-, *m*- and *p*-cresols.

TABLE I

NITRATION PRODUCTS OF THE CRESOLS PREPARED ACCORDING TO THE NOLTING AND SALIS REACTION

Serial No.	Cresols or mixture	Yield		Melting point °C.		Analysis for once recrystallised material						Empirical formula	Identity
						Calculated:			Found:				
		Cresol/product g.	per cent.	Crude	Once recrystallised	C per cent.	H per cent.	N per cent.	C per cent.	H per cent.	N per cent.		
1	*o*-Cresol	27/36·5	73	87·0	86·0	42·4	3·03	14·1	42·9	3·2	13·7	$C_7H_6N_2O_5$	4:6-Dinitro-*o*-cresol (*d*)
2	*m*-Cresol	27/26·5	44	104·5	106·0	34·5	2·05	17·2	35·2	2·3	16·7	$C_7H_5N_3O_7$	2:4:6-Trinitro-*m*-cresol, Cresylite (*d*)
3	*p*-Cresol full nitration (*a*)	27/5·0	10	78·0	80·0	42·4	3·03	14·1	42·9	3·3	13·8	$C_7H_6N_2O_5$	Both products are 2:6-dinitro-*p*-cresol, Victoria Yellow (*d*)
4	limited nitration	27/16·5	33	79·5	81·0	42·4	3·03	14·1	43·0	3·04	13·4		
5	8:1:1 *o*-, *m*-, *p*-cresols "80 per cent." mixture (*b*)	100/99	(*b*)	78·5 (*d*)	79·5	—	—	—	—	—	—	—	Approximately: 85 per cent. of dinitro-*o*-cresol; 10 per cent. of trinitro-*m*-cresol; 5 per cent. of dinitro-*p*-cresol
6	1:1:1 *o*-, *m*-, *p*-cresols "33 per cent." mixture (*b*)	30/12	(*b*)	40-<50	—	—	—	—	—	—	—	—	Approximately: 60 per cent. of dinitro-*o*-cresol; 30 per cent. of trinitro-*m*-cresol; 10 per cent. of dinitro-*p*-cresol (*c*)

NOTES:

(*a*) Full nitration carried out at 105° C.; limited nitration at lower temperature (50° C.).

(*b*) Mixed products have "mixed" molecular weights so percentage yield is not given.

(*c*) Different runs gave varying yields according to the dilution (high dilution—5 to 10-fold—high yield and *vice versa*) and temperature of reaction (when maintained at about 105° C.—lower yields).

(*d*) See Table VI for ultra-violet absorption spectra data.

to mice by intraperitoneal injection and sacrificing equal groups at 4 and 24 hours later in order to obtain the blood concentrations of the nitro-cresols. The concentrations of the compounds in blood were estimated essentially by the method of Parker[16] for serum dinitro-*o*-cresol as modified by Harvey[17] and by King and Harvey[18] for dinitro-*o*-cresol in whole blood of man and of animals. Minimum transmission values were obtained for the sodium salts of the compounds in methyl ethyl ketone at appropriate wavelengths (see Table III, last column, and Fig. 3).

Application of the Nolting and Salis reaction to o-, m- and p-cresols

The nitration of the three cresols by the method of Nolting and Salis results in three main compounds, dinitro-*o*-cresol from *o*-cresol, dinitro-*p*-cresol from *p*-cresol and trinitro-*m*-cresol from *m*-cresol (Table I).

Although limited quantities of sulphuric acid were used in the sulphonation of *o*-cresol (Datta and Mitter, *loc. cit.*, Claus and Jackson, *loc. cit.*), subsequent treatment of the product with nitric acid appeared to result in 4:6-dinitro-*o*-cresol and no 5:6-dinitro-*o*-cresol could be identified (cf. Datta and Varma, *loc. cit.*). Preliminary steam distillation to remove any unchanged *o*-cresol reduced the yield of dinitro-*o*-cresol in the mother liquor, but treatment of the steam distillate, containing unchanged *o*-cresol, with nitric acid at 100° C. for 8 hours and for 7 days at room temperature yielded further quantities of dinitro-*o*-cresol (Table II). Excessive dilution (>10-fold) of the sulphonated mixture made it necessary to heat the nitration mixture, but if dilution was about 3-fold the reaction became too violent, cooling was necessary and some product was completely destroyed. The formation of trinitro-*m*-cresol required that the mixture should be raised to about 105° C., otherwise the yield of separated material was low. *p*-Cresol was readily nitrated (*vide supra*), and it was found that cautious addition of nitric acid to the sulphonated mixture caused the precipitation of a bulky mass of yellow crystals at relatively low temperatures (less than 50° C.). On recrystallisation this product proved to be identical with a recrystallised commercial specimen of 2:6-dinitro-*p*-cresol. If the temperature was allowed to exceed 80° C. the reaction became violent and much of the yellow separated dinitro-*p*-cresol was destroyed, leaving only a small yield of the substance. Table III summarises some properties of the nitro-cresols.

Separation of the nitro-cresols: a simple test for their differentiation

Benzene solutions of the nitro-cresols or their mixtures gave definite colour bands on benzene pre-treated alumina columns. Three colour bands were formed: top layer—bright yellow trinitro-*m*-cresol, middle layer—orange-red dinitro-*p*-cresol, and the lowest band yellow dinitro-*o*-cresol. Some commercial samples (Harvey, *loc. cit.*) gave a small indefinite layer of brown material on top of the column, but this was not further investigated. Analysis of the extracts of the three colour bands yielded substances which were impure (intermixed) specimens of trinitro-*m*-cresol, dinitro-*p*-cresol and dinitro-*o*-cresol (Fig. 2). The melting points were low with the exception of those obtained for dinitro-*o*-cresol

TABLE II

THE NITRATION OF *o*-CRESOL

	Conditions of sulphonation			Pre-nitration treatment (*a*)	Product yield per cent.	Melting point °C.		Probable identity of product (*d*)
	mols. H_2SO_4	Temperature °C.	Duration: hours			Crude	Once recrystallised from ethanol	
1	1·2	Cold 0 to 4	24	Steam distillation	21	87·5	87·5	4:6-Dinitro-*o*-cresol
2				Dilution	66	84·5	87·0	,,
3	1·2	Hot: boiling water bath 100	10	Steam distillation	74	86·5	86·0	,,
4				Dilution	77	87·5	86·0	,,
5	2·2	Hot: boiling water bath 100	2	(i) Steam distillation	8	—	87·5	,, (*c*)
6				(ii) Steam distillate from (i) (*b*)	25	—	87·5	,,
7				(iii) Dilution	50–70	—	87·5	,,

NOTES:

(*a*) With the exception of (*b*) below only mother liquors were nitrated.

(*b*) The steam distillate, 200 ml., was treated with concentrated nitric acid, A.R., 100 ml. heated on boiling water bath for 8 hours, allowed to stand at room temperature for 7 days before separating off the product and recrystallising.

(*c*) Analysis figures for the 3 products are as follows:

(i) Steam distillation (mother liquor); Found: C, 42·4; H, 3·1; N, 13·6 per cent.
(ii) Steam distillate from (i) C, 42·1; H, 3·3; N, 13·8 per cent.
(iii) Dilution C, 42·6; H, 3·1; N, 13·7 per cent.
Calculated for $C_7H_6N_2O_5$ C, 42·4; H, 3·03; N, 14·1 per cent.

(*d*) See Table VI for ultra-violet absorption spectra data.

that were as high as any recorded, and these specimens appeared to be fairly pure. Such colour bands did not occur when chromatographic cellulose powder was used. The explanation of this probably lies in the fact that some salts (e.g., sodium) of the nitro-cresols studied have characteristic colours, and the obvious salts in this separation would be those formed by reaction with aluminium oxide.

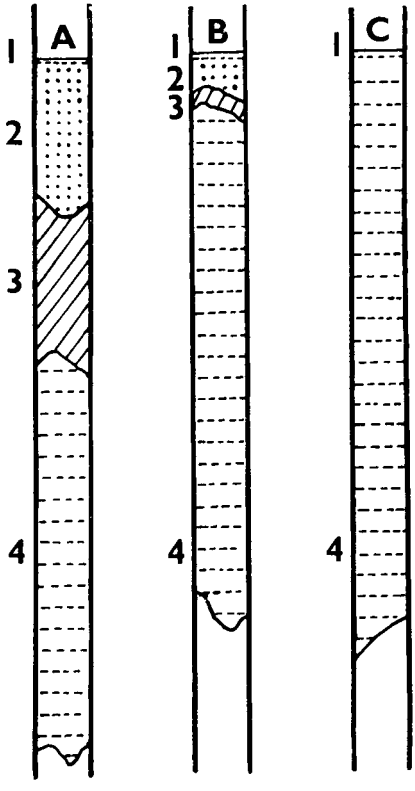

FIG. 2. Absorption of dinitro-*o*-cresol, trinitro-*m*-cresol and dinitro-*p*-cresol on alumina:—

A. From 33 per cent. mixture. 1. Dark brown. 2. Bright yellow. Mainly trinitro-*m*-cresol, m.pt. (recryst.) 95° to 97° C.; found, N = 16·2 per cent.; Calc., N = 17·2 per cent. 3. Orange-red. Mainly dinitro-*p*-cresol, m.pt. (recryst.) 62° C.; found, N = 14·5 per cent.; Calc., N = 14·1 per cent. 4. Yellow. Mainly dinitro-*o*-cresol, m.pt. (recryst.) 87·5° C.; found, N = 14·6 per cent.; calc., N = 14·1 per cent.

B. From 80 per cent. mixture. 1. Dark brown. 2. Trinitro-*m*-cresol. 3. Dinitro-*p*-cresol. 4. Yellow. Mainly dinitro-*o*-cresol, m.pt. (recryst.) 87·5° C.: found, N = 15·3 per cent.; calc., N = 14·1 per cent.

C. From commercial dinitro-*o*-cresol. 1. Dark brown. 4. Yellow. Dinitro-*o*-cresol m.pt. (recryst.) 87·5° C.: found, N = 14·5 per cent.; calc., N = 14·1 per cent.

This differentiation of the colour bands might prove to be useful initially in testing the purity of a dinitro-*o*-cresol sample when contamination by trinitro-*m*-cresol or by dinitro-*p*-cresol is suspected. For example, it was found that 25 ml. of a benzene solution containing 249 mg. of dinitro-*o*-cresol and 1 mg. of contaminating dinitro-*p*-cresol or trinitro-*m*-cresol was readily differentiated into two coloured bands on an alumina column approximately 9 mm. in diameter.

The depths of the bands are an approximate indication of the proportionate concentration of each substance present. Obviously this cannot be accepted as an accurate measure since the flow rates of the three substances are different and are in the order of speed, dinitro-*o*-cresol, dinitro-*p*-cresol, trinitro-*m*-cresol. The depths of the bands increased disproportionately on continued elution.

No other nitro compounds have been mixed deliberately with the 3

TABLE III

SOME PROPERTIES OF THE THREE NITRO-CRESOLS

Substance	Appearance	Solubility in water (*a*) per cent.	Melting point ° C.	Colour 10 cm. depth of 1 per cent. alkaline solution (Na, Al, etc.)	Polarogram values Halfwave potential of 10 μg./ml. in 0·1N sodium hydroxide (v.)	Minimum transmission in visible spectrum (*e*) mμ
Dinitro-*o*-cresol ..	Yellow needles or rhombohedrons	0·018 to 0·024 (*b*)	86·5 to 87·0	Yellow-orange	−0·530	430
Trinitro-*m*-cresol ..	Pale yellow needles	0·094	106 to 109	Bright yellow	−0·550 (*c*)	415
Dinitro-*p*-cresol ..	Yellow to yellow-brown needles or rhombohedrons	0·029	80 to 81	Red-orange	−0·455 to −0·475 (*d*)	475

NOTES:
(*a*) Determined by shaking up solid material in water at 18° C. and estimating the dissolved material by Parker's method.
(*b*) See Harvey[1].
(*c*) At 10 μg./g. there was a tendency to form several waves.
(*d*) The values obtained are −0·455 v. for commercial sample and −0·475 v. for sample prepared by limited nitration.
(*e*) See Figure 3 for visible absorption spectra, Figure 4 for ultra-violet absorption spectra and Table VI for ultra-violet absorption spectra data.

TABLE VI

ULTRA-VIOLET SPECTROGRAPHIC DATA OF THE NITRO-CRESOLS (M/100 SODIUM HYDROXIDE)

Substance	Reference	Ultra-violet spectrographic data					
		λmax. mμ	*E* mol. × 10^{-4}	λmax. mμ	*E* mol. × 10^{-4}	λmax. mμ	*E* mol. × 10^{-4}
Dinitro-*o*-cresol, pure (authentic) ..	—	370	1·52	263	0·72	222	1·20
,, (Noting and Salis) ..	Serial No. 1, Table I	370	1·53	265	0·76	218	1·17
,, (cold sulphonation) ..	Serial No. 1, Table II	368	1·10	262	0·48	225	0·71
,, (cold sulphonation) ..	Serial No. 1, Table II	368	1·39	263	0·64	225	0·74
Dinitro-*p*-cresol, pure (authentic) ..	—	446	0·64	259	0·63	225	1·84
,, (full nitration)	Serial No. 3, Table I	446	0·68	259	0·66	225	1·90
,, (part nitration) ..	Serial No. 4, Table I	446	0·70	259	0·67	225	1·93
Trinitro-*m*-cresol, pure	Serial No. 2, Table I	348	1·21	260	0·848	215	1·44

studied in this communication. Also, it is obvious that the presence of additional contaminants cannot be excluded completely. However, examination of melting points, and the ultra-violet absorption spectra data suggests that it is unlikely that such substances, if they are formed, will occur in significant quantities.

Physiological tests

LD50 values for pure dinitro-*o*-cresol and for pure dinitro-*p*-cresol were similar and contamination of the former with the latter or with trinitro-*m*-cresol caused relatively little reduction in the toxicity or any obvious changes in the symptoms (Table IV). The symptoms exhibited

TABLE IV

LD50 VALUES AND SOME PHYSIOLOGICAL EFFECTS OF DINITRO-*o*-CRESOL, TRINITRO-*m*-CRESOL, DINITRO-*p*-CRESOL AND THEIR MIXTURES

Substance (*a*)	LD50 mg./kg.	Effects
Pure dinitro-*o*-cresol	24·2	Sweating, increased respiration, thirst, "stretching" of the body, diminished physical activity, death accompanied by marked rigor. Fully described by many authors
"80 per cent." Dinitro-*o*-cresol (*b*)	22·9	Ditto
"33 per cent." Dinitro-*o*-cresol (*b*)	32·5	Ditto
Dinitro-*p*-cresol	24·8	Ditto
Trinitro-*m*-cresol	168·0	Animals tend to "bunch" up, hair becomes erect giving a porcupine-like appearance, marked "shivering" or fibrillating movements, occasional spasms followed by great nervous activity, i.e., running very rapidly round the cage. Death not accompanied by any marked rigor

NOTES:
(*a*) Given as 0·5 per cent. solutions in 0·5 per cent. NaCl, 0·5 per cent. $NaHCO_3$ by intraperitoneal injection.
(*b*) Prepared by nitrating mixtures of 8:1:1 and 1:1:1 *o*-, *m*- and *p*-cresols (see Table I).

by animals poisoned by dinitro-*o*-cresol and dinitro-*p*-cresol were different from those poisoned by trinitro-*m*-cresol. The marked "fibrillation" or shivering resulting from trinitro-*m*-cresol was in marked contrast to the reduction of physical activity caused by the dinitro-cresols. Blood levels of the three substances taken at 4 and 24 hours following dosing gave similar reduction values, i.e., 76 to 82 per cent. (Table V).

TABLE V

ELIMINATION OF NITRO-CRESOLS BY MICE

Substance (*a*)	Blood levels μg./g.		Reduction over 4 to 24 hours per cent.
	4 hours after injection	24 hours after injection	
Dinitro-*o*-cresol (*b*) ..	33, 36, 43, 35 Mean 37 ± 4·5	5, 8, 5 Mean 6 ± 0·6	80
Trinitro-*m*-cresol ..	66, 60, 46, 60, 64, 54 Mean 58 ± 7·3	12, 12, 10, 12, 14, 1 Mean 10 ± 4·6	82
Dinitro-*p*-cresol ..	31, 32, 38, 53, 27, 28 Mean 35 ± 8·9	8, 7, 9, 7, 8, 8 Mean 8 ± 0·32	75

NOTES:
(*a*) Given as single intraperitoneal injection of 20 mg./kg. of substance in 0·5 per cent. NaCl, 0·5 per cent. $NaHCO_3$ solution.
(*b*) Figures from a wider investigation which will be reported elsewhere.
(*c*) Means (± S.D.) to nearest μg.

Spectrum analysis of the nitro-cresols

Absorption curves in the visible spectrum were prepared by examining the sodium salts of the nitro-compounds in methyl ethyl ketone at concentrations of 5 μg./ml. These are shown in Figure 3. Transmissions at the minimum values were used in the estimation of the 3 compounds in blood (dinitro-*o*-cresol 4300 Å., trinitro-*m*-cresol 4150 Å. and dinitro-*p*-cresol 4750 Å.).

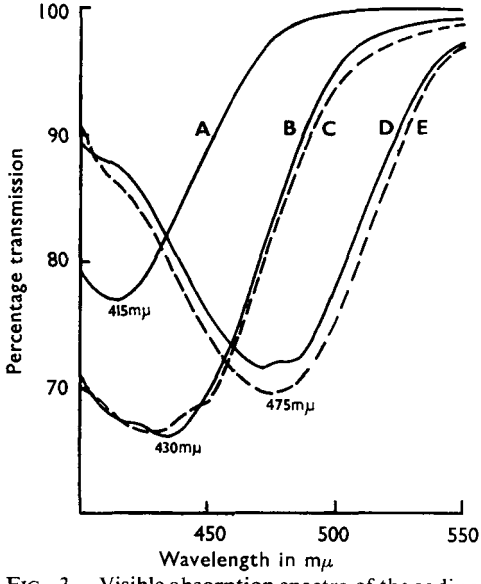

FIG. 3. Visible absorption spectra of the sodium salts of dinitro-*o*-cresol, trinitro-*m*-cresol and dinitro-*p*-cresol. All in methyl ethyl ketone.
A. Trinitro-*m*-cresol (recrystallised).
B. Dinitro-*o*-cresol (crude).
C. Dinitro-*o*-cresol (recrystallised).
D. Dinitro-*p*-cresol (once recrystallised).
E. Dinitro-*p*-cresol (twice recrystallised).

Ultra-violet absorption spectra of dinitro-*o*-cresol, trinitro-*m*-cresol and dinitro-*p*-cresol are shown in Figure 4. Details of the ultra-violet spectra of authentic compounds and of other substances prepared in the course of this investigation are given in Table VI.

Molnar, *loc. cit.*, has recorded curves of several nitro-compounds, including his unidentified Dinitro-cresols I and II. Summarised comparisons between Molnar's values and those obtained in the present investigation are as follows:

	Maximum value mμ	
	Molnar	Present investigation
Dinitro-*o*-cresol, pure	—	368–70
Dinitro-cresol I	360	—
Dinitro-*p*-cresol, pure	440	446
Trinitro-*m*-cresol	360	348

There is good agreement between Dinitro-cresol I and pure dinitro-*o*-cresol and reasonable agreement between the dinitro-*p*-cresol values except in the shorter wavelengths. Agreement is not good between the trinitro-*m*-cresol samples. No curves have been obtained in the present investigation that correspond precisely to Molnar's Dinitro-cresol II. It is interesting to note that the molar extinction coefficients of the second two samples of dinitro-*o*-cresol (see Table VI) are low, although the forms of their spectra are indistinguishable from the authentic specimen obtained essentially by the method of Nolting and Salis (see Tables I

and II). Dinitro-*p*-cresol specimens prepared by limited nitration at low temperatures or by full nitration at higher temperatures give spectra which are identical with authentic recrystallised dinitro-*p*-cresol.

No explanation can be advanced for the low molar extinction coefficients of the second two dinitro-*o*-cresols (Table VI), but the similarity of the spectral forms of all four compounds leaves little doubt that the identity of these compounds is 4:6-dinitro-*o*-cresol.

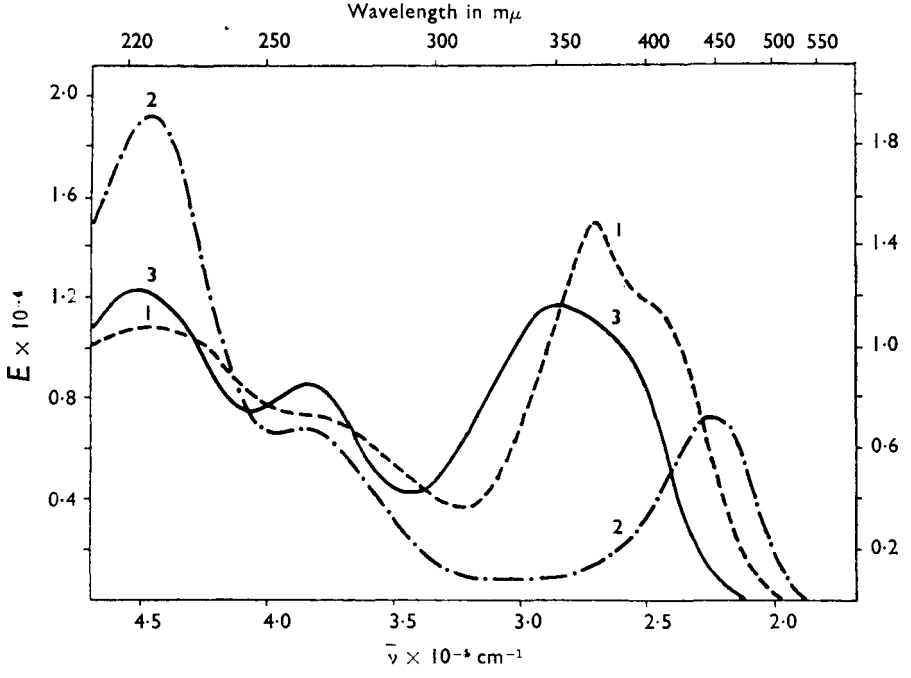

FIG. 4. Absorption spectra of dinitro-*o*-cresol, trinitro-*m*-cresol and dinitro-*p*-cresol. Solvent 0·01N sodium hydroxide.

1. 4:6-Dinitro-*o*-cresol.
2. 2:6-Dinitro-*p*-cresol.
3. 2:4:6-Trinitro-*m*-cresol.

DISCUSSION

If the nitration of *o*-cresol is carried out in the presence of excess of sulphuric acid as a nitrating agent, 4:6-dinitro-*o*-cresol is the only product. Provided that the *o*-cresol used is a good grade, significant contamination by the *m*- and *p*-isomers is unlikely. Nevertheless, if *p*-cresol is present 2:6-dinitro-*p*-cresol may contaminate the final product to a minor extent, but if the temperature has been maintained above 80° C., then it is likely that the final yield of dinitro-*p*-cresol will be low since it is destroyed in the presence of excess of nitric acid or nitrous fumes at high temperatures.

It is clear that fairly wide limits in the experimental conditions can be observed in the preparation of dinitro-*o*-cresol without causing any major changes in its yield or purity. In any case, if the reaction is carried

out at high temperature, i.e., >80° C., the vigorous conditions will favour destruction of formed substances rather than the formation of a new one. This is illustrated by the preparation of dinitro-*p*-cresol.

It is interesting to compare this with the other findings of Datta and Varma (*loc. cit.*), who have stated that excess of nitrous gases on *p*-cresol sulphonate gives rise to some oxalic acid and practically no nitro-compounds, and that the presence of free (unsulphonated) cresol causes the formation of much tarry material.

The chromatographic separation of the three nitro-compounds could serve as the basis for a simple colour test in checking the purity of samples of dinitro-*o*-cresol when contamination by dinitro-*p*-cresol or trinitro-*m*-cresol is suspected, since its sensitivity is 0·5 per cent. or less on a few mg. of crude material. Because this method has not been extended to other nitro-cresols their presence cannot be excluded, although the slightly lowered melting points of the second and third groups of the preparations recorded in this paper suggest a general homogeneity of substance.

Although Molnar's Dinitro-cresol I and pure dinitro-*o*-cresol have similar, if not identical ultra-violet absorption curves, the melting points are at variance (Molnar gives 105° C. for Dinitro-cresol I and 85·5° C. for Dinitro-cresol II). The value of 105° C. corresponds most closely to 2:6-dinitro-*m*-cresol, melting point 101° C. (Will[20]), or 4:6-dinitro-*m*-cresol, melting point 97° C. (Gibbs and Robertson[21]), both of which are prepared by indirect means. If *m*-cresol was a contaminant in the preparation of the two Dinitro-cresols reported by Molnar, then trinitro-*m*-cresol, and not a dinitro-*m*-cresol, would be the resulting compound.

It has been stated by Oetlingen[19] that dinitro-*o*-cresol is sometimes contaminated by dinitro-*p*-cresol. The experiments reported in this paper suggest that dinitro-*p*-cresol is about as toxic as dinitro-*o*-cresol towards mice. The visible symptoms of animals treated with the two compounds are very similar. Therefore any minor contamination is unlikely to reduce the gross toxic effect. However, examination of several commercial samples manufactured in this country (Harvey, *loc. cit.*) did not reveal any contaminating dinitro-*p*-cresol. The alumina test appeared to be quite sensitive and there was no suggestion of any orange-red band dinitro-*p*-cresol appearing on the columns used for the analysis of the commercial samples. If any trinitro-*m*-cresol is present in the sample it will not lower the effective total toxicity to any extent. It is interesting to record that a "33 per cent." sample, containing about 30 per cent. of trinitro-*m*-cresol is about 60 per cent. less toxic than pure dinitro-*o*-cresol, wheras trinitro-*m*-cresol itself has about 12 per cent. of the toxicity of pure dinitro-*o*-cresol. This suggests that trinitro-*m*-cresol does not depress the toxicity of the other two compounds to any extent. All 3 compounds appear to be hyperthermic, and according to Molnar the dinitro-cresols are more toxic and hyperthermic than *o*-nitro-*p*-cresol and 2:4:6-trinitro-*m*-cresol. The identity of the two dinitro-cresols described by Molnar has not been established, although one compound has some properties similar to dinitro-*o*-cresol. They

appear to be metabolic stimulants and produce similar toxicological effects, although to a differing degree.

The differences in the symptoms of the 3 compounds are interesting and possibly significant, and the similar rates of their elimination from mice suggest that in other species there is the possibility of some cumulative action of dinitro-*p*-cresol and trinitro-*m*-cresol. Arguing by analogy with the action of dinitro-*o*-cresol (King and Harvey[22]) it is likely that trinitro-*m*-cresol and dinitro-*p*-cresol will accumulate in man if absorbed in small quantities at frequent, i.e., daily, intervals. Thus, in manufacturing or using dinitro-*p*-cresol or trinitro-*m*-cresol, the same rigorous safety precautions should be adopted as already recommended for handling dinitro-*o*-cresol (Hunter[23]).

Summary and Conclusions

1. The preparation of 4:6-dinitro-*o*-cresol by the method of Nolting and Salis has been studied and it has been demonstrated that if pure *o*-cresol is sulphonated by limited or excess quantities of sulphuric acid, and nitrated by excess of nitric acid at temperatures $>80°$ C., only dinitro-*o*-cresol will result.

2. Dinitro-*p*-cresol and trinitro-*m*-cresol are likely to be formed if the *o*-cresol is contaminated by the *m*- and *p*-isomers.

3. Dinitro-*o*-cresol and dinitro-*p*-cresol appear to be equally toxic to mice and result in similar symptoms.

4. Trinitro-*m*-cresol has about 12 per cent. of the toxicity of dinitro-*o*-cresol and results in different symptoms.

5. A few properties of dinitro-*o*-cresol, dinitro-*p*-cresol and trinitro-*m*-cresol have been summarised.

6. Attention has been drawn to the use of alumina for separating and identifying the 3 nitro-compounds studied. This has been suggested as a simple preliminary check for testing the purity of dinitro-*o*-cresol samples.

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