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Some Observations on the Absorption and Excretion of 4:6-Dinitro-*o*-cresol (DNOC)

1. BLOOD DINITRO-*o*-CRESOL LEVELS IN THE RAT AND THE RABBIT FOLLOWING DIFFERENT METHODS OF ABSORPTION

BY E. KING AND D. G. HARVEY

Department for Research in Industrial Medicine, Medical Research Council, M.R.C. Laboratories, Hampstead, and the London Hospital, E. 1

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The widespread use of 4:6-dinitro-*o*-cresol* (DNOC) in Great Britain and elsewhere, and the fatal casualties caused by its use amongst agricultural workers have been fully reported by Bidstrup & Payne (1951). A great deal is known about its toxicity (Ambrose, 1942; Spencer, Rowe, Adams & Irish, 1948; Parker, Barnes & Denz, 1951), but no attention has been paid to the possible significance of blood DNOC levels resulting from natural methods of absorbing the substance.

It appeared to us to be essential to rectify this omission for two main reasons. First, a study of blood DNOC levels would give some indication of the possible cumulative effect of DNOC. Secondly, a correlation of blood DNOC levels with early symptoms might be used in a 'monitoring' system to safeguard workers exposed to DNOC. Obviously it was essential to plan some trials on the absorption of

DNOC by human volunteers but before these could be undertaken it was necessary to gain further information from animal experiments on the absorption of DNOC, the significance of the resulting blood levels and the effect of environmental temperatures.

The results of the human trials have now been published (Harvey, Bidstrup & Bonnell, 1951). They have been correlated with environmental studies in the field (Bidstrup, Bonnell & Harvey, 1952) and certain practical recommendations have been made (Harvey, 1952).

The present communication reports the results of the first group of animal experiments, the detailed objects of which were to investigate: (a) the significance of blood DNOC levels following administration of one or more daily doses of DNOC after its absorption through the alimentary canal, the lungs and through the skin of rats and of rabbits; (b) the effect of environmental temperatures on the blood DNOC levels, body temperature, respiratory rate and mortality of the rat and the rabbit.

* 3:5-Dinitro-*o*-cresol in the usage of the Chemical Society, 4:6-dinitro-*o*-cresol in the usage of the American Chemical Society or, systematically, 2-methyl-4:6-dinitrophenol.

EXPERIMENTAL METHODS

The experiments described were carried out with the technical assistance of Miss Jean Peal and Mr K. E. Carling.

DNOC. All samples were recrystallized from aqueous ethanol before use. Solutions of 0.1, 0.2, 0.5 and 2.0% (w/v) were made by dissolving crystalline DNOC in physiological (0.9%) saline containing the appropriate amount of NaOH or NaHCO_3 .

sprays, but the results appear unreliable as the rats were allowed to lick their bodies.

Preliminary experiments indicated that it was difficult to produce from a solution or suspension of DNOC or of its salts an aerosol comparable to that formed during actual spraying operations. It was therefore decided to produce a sublimate of DNOC which would act as an aerosol. The apparatus used (Fig. 1) consists essentially of a large all-glass tank capable of holding a small cage for six rats. In the

Fig. 1. Apparatus for producing DNOC aerosol.

Animals. Albino and hooded rats, albino, Himalayan and chinchilla rabbits were used throughout. Groups of four to twelve animals were normally employed, except in a few experiments with single animals. There appeared to be some suggestion of species difference in behaviour towards DNOC. Rats were fed on unlimited quantities of rat diet 41, and water, with weekly supplements of butter and milk. Rabbits were fed on bran, oats, water and greens with regular supplements of cod-liver oil.

Sampling. Rats were bled from the tail and rabbits from the ear into a weighed tube containing a known amount of heparin solution. In all cases samples of 0.1–0.5 g. were obtained.

Method of analysis. The rapid and accurate method of Parker (1949) was used throughout the investigations. Colorimetric measurements were made on a Unicam D.G. spectrophotometer, a Spekker absorptiometer and a Gallenkamp Monocell absorptiometer.

Methods of administration of DNOC

Ingestion. Although Ambrose (1942) and Spencer *et al.* (1948) incorporated DNOC in the diets of their animals, it was felt that more satisfactory results would be obtained by giving DNOC by stomach tube. This would ensure that an exact amount entered the alimentary canal, thus eliminating the time factor taken up by feeding, and also the risk that unequal amounts might be ingested by the animals. Light ether anaesthesia was used when DNOC was administered.

Inhalation. The only reference to the inhalation of DNOC appears to be that of the Experimental Station at Suffield, Alberta (1947), where rats were exposed to settling DNOC

tank is a thermostatically controlled electric apparatus, known as an Aerovap (manufactured by Shepherd's Aerosol Ltd.) which is capable of subliming DNOC at a steady rate.

Fig. 2. Photomicrograph of DNOC aerosol crystals. The largest crystal in centre of field is approximately 15μ . in length.

An air-flow of 1 l./min. was passed over the top of the Aerovap. The air-flow was measured by means of a Rotameter, and governed by an adjustable leak in the usual way.

At a temperature of $130 \pm 2^\circ$ DNOC sublimed from the Aerovap at the rate of 0.2 mg./hr. Some attempt was made to carry out a size-frequency count on the particles of DNOC, but it was found that the hot wire of the Thermal Precipitator used for this assessment tended to fracture the crystals. The crystals of DNOC from the aerosol, which settled on a glass slide placed in the tank during 30 min. following cut-off of the air-flow, are illustrated in Fig. 2; the maximum length of the crystals is about 15μ . and there are many shorter than 3μ . A constant check on the concentration of air-borne DNOC was made in the following manner. The outflow of the tank was scrubbed by passing it into a long glass tube, 50×5 cm., loosely packed with glass wool wetted with 5% (w/v) aqueous NaOH. The tube was well washed out with dilute alkali and the DNOC estimated in the usual way. An average 10 min. run under the conditions stated gave a mass concentration of 100 mg. DNOC/cu.m.

Passage of the DNOC-laden air into 10–20 ml. of dilute aqueous NaOH, contained in an ordinary flask, was not adequate to remove all the DNOC. Thus it was necessary to use the more cumbersome but more efficient method of scrubbing when making routine checks of mass concentration.

The purity of the sublimate was checked by determining its melting point. This was identical with that obtained for pure DNOC. Addition of DNOC sublimate to pure DNOC did not cause a depression of the melting point.

Skin application. Both Ambrose (1942) and Spencer *et al.* (1948) have given some information on the absorption through the skin of DNOC in aqueous solution and also in organic solvents; they have deduced lethal doses therefrom but have not determined blood DNOC levels. In the present investigation only rabbits were used, because it was found to be practically impossible to prevent rats from licking their bodies after skin application of DNOC. The rabbits were shaved in the thoracico-lumbar region of the back 24 hr. before application of DNOC to the skin. In the first series of experiments DNOC was applied as an aqueous solution of its sodium salt, either as 5 ml. of a 1% solution covering 100 sq.cm., or as 5 ml. of a 2% solution covering 50 sq.cm. In the second experiment 2% aqueous DNOC was sprayed evenly over a shaved area to give a concentration of 5 mg./g. body weight.

Intraperitoneal injection. This method was used in order to make comparison of results with those obtained from the natural methods of administration.

Dose. The least dose administered was 1 mg./kg., but for the majority of experiments 5 mg./kg. was taken as a minimal dose.

RESULTS

These are set out in Tables 1–7 and in Figs. 3–8.

The rate of absorption of DNOC from the alimentary canal of the rat is illustrated in Table 1. Analysis of the alimentary canal, its contents and the blood indicates that only 20% of a single large dose of DNOC can be recovered 1 hr. after administration, about 10% after 2 hr., and 5% after 7 hr. The quantity of DNOC retained in the large intestine is small but remains fairly constant ($5\text{--}26\mu\text{g.}$, average $12.5\mu\text{g.}$). Forty-eight hours after dosing there are still significant quantities of DNOC present in the large intestine, even though it has practically disappeared from the gut contents and from the blood of the animal. It has not been determined whether the DNOC in the large intestine identified at this late stage after administration is due to continued absorption from the gut or to excretion from the blood.

The DNOC in the blood of rats and of rabbits appears to reach a maximum value 2–4 hr. after administration of a single dose by stomach tube (Table 2). In contrast, the blood DNOC levels following intraperitoneal injections increase more rapidly to reach maximum values which are usually a good deal higher than those resulting from identical doses administered by stomach tube. In general, 24 hr. after administration of single doses of DNOC in the range $5\text{--}50$ mg./kg., the blood DNOC levels fall to about $5\text{--}25\mu\text{g./g.}$ Doses of 1 mg./kg. give very low blood DNOC levels which remain low. Administration of DNOC by stomach tube to the

Table 1. *Absorption of DNOC following single dose of 30 mg./kg. given by stomach tube to albino rats*

(Rats bled and then killed immediately by ether; entire gut removed; contents well washed out; tissues and contents homogenized before analysis.)

Dose (mg.)	Time after dose (hr.)	DNOC ($\mu\text{g.}$)					Total DNOC	
		Blood ($\mu\text{g./g.}$)	Stomach	Small intestine	Large intestine	Contents of alimentary canal	(mg.)	% of dose
4.14	1	34.0	110.0	13.0	11.0	660.0	0.79	19.2
6.00		30.0	292.0	10.0	5.0	800.0	0.11	18.5
4.62	2	60.5	55.0	16.3	20.0	450.0	0.54	11.7
5.01		45.5	61.0	13.0	8.0	540.0	0.62	12.4
4.65	4	36.5	22.0	13.0	7.0	92.0	0.13	2.9
4.62		29.3	26.0	12.0	6.0	124.0	0.17	3.6
4.89	7	57.2	33.0	13.0	26.0	114.0	0.18	3.8
3.60		52.0	33.0	13.0	12.0	18.0	0.07	2.1
4.86	24	22.5	5.0	11.0	Lost	8.0	>0.02	>0.5
3.90		25.0	6.0	11.0	17.0	6.0	0.04	1.0
3.45	48	3.0	1.0	4.0	12.0	7.0	0.02	0.7
3.66		2.8	1.0	3.0	14.0	7.0	0.02	0.7

Table 2. *Blood DNOC levels in animals following single doses*

(ST, administration by stomach tube; IP, intraperitoneal injection. All animals survived dosage except two rats, 20 and 100 mg./kg. For further information on 30 and 40 mg./kg. see Tables 1 and 8. Each experiment was on a single animal except for the 30 mg./kg. (two rats) and 40 mg./kg. (twelve rats); for the latter group mean $\pm$ s.e. is given.)

Dose (mg./ kg.)	Admin.	DNOC (μ g./g.)											
		Time after dose (hr.)											
		1	2	3	4	5	6	7	8	11	12	24	27
Rats													
1	Oral	2.5	—	3.3	—	3.8	—	—	2.9	—	—	3.7	—
5	ST	8.8	—	13.0	—	9.3	—	—	—	—	—	12.0	—
10	ST	48.0	—	16.0	—	26.0	—	—	—	—	—	25.0	—
20	ST	46.0	—	17.0	—	48.0	—	—	—	—	—	—	—
30	ST	34.0	60.5	—	36.5	—	—	57.2	—	—	—	22.5	—
		30.0	45.5	—	29.3	—	—	52.0	—	—	—	25.0	—
40	ST	—	100.3 $\pm$ 1.98	—	100.5 $\pm$ 2.85	—	97.4 $\pm$ 1.53	—	—	—	—	—	—
50	ST	—	—	58.0	60.0	—	—	—	92.0	—	80.0	19.0	—
100	ST	—	—	85.0	88.0	—	—	—	—	—	—	—	—
1	IP	4.7	—	7.1	—	5.0	—	—	4.9	—	—	—	—
5	IP	8.8	—	14.1	—	15.7	—	—	23.8	12.2	—	—	9.8
10	IP	28.4	—	40.5	—	33.7	—	—	34.7	30.6	—	—	15.1
20	IP	101.0	—	97.0	—	63.2	—	—	64.6	43.5	—	—	25.8
Rabbits													
5	Oral	—	8.1	—	6.4	—	10.0	—	20.0	—	4.6	7.4	—
10	ST	—	24.0	—	44.0	—	20.0	—	18.0	—	5.5	4.0	—
20	ST	—	24.0	—	31.0	—	31.0	—	8.2	—	18.8	2.5	—

rabbit results in a similar build-up of the blood DNOC levels over the first 4–6 hr., but the values obtained are much lower than those resulting in the rat from similar doses. Twenty-four hours later the blood DNOC values are reduced to 0–7 μ g./g.

When repeated daily doses of DNOC are administered to the rat by stomach tube or by intraperitoneal injection they accumulate to a limited extent. This accumulation appears to take place in two stages. First, two daily doses of DNOC (5–20 mg./kg.) result in a blood DNOC level which is significantly higher than that resulting from a single daily dose. Secondly, continuation of the doses after the second day maintains the 2-day level at more or less constant values, although there may be some small but significant deviations. The increases in blood DNOC levels between days 2 and 3 (D + 2 and D + 3) are rarely significant. The results of the experiments leading to these observations are given in Table 3. Only two groups of animals do not show a significant increase in the blood DNOC levels from D + 1 to D + 2. They are the rabbits receiving 25 mg./kg. by stomach tube, and the six albino rats receiving 5 mg./kg. by intraperitoneal injection.

Of the seven groups of animals studied, only two have significant mean slopes from the third day onwards. These are the two groups of hooded rats given 5 and 10 mg./kg. by stomach tube. The slopes are positive and have mean values of about 1.3 μ g./g./day. They are small in comparison with the mean

blood DNOC values on the second day which are 10.0 and 24.0 μ g./g. respectively.

Although there are no other significant mean slopes some individual animals show significant positive and negative slopes. The values of these are usually very small and rarely exceed 1 μ g./g./day.

Individual slopes with significant negative values are found chiefly in three groups, namely the rats to which large doses (20 mg./kg.) have been given orally, and the two groups of rats given 5 and 10 mg./kg. by intraperitoneal injections.

A further effect of repeated daily doses of DNOC is shown by the weight curves on Table 4 and Fig. 3. Although ten daily doses of 5 mg./kg. do not appear to alter the growth rate of the animals, the same number of doses of 10 and 25 mg./kg. causes significant reductions in body weight. Restoration of growth appears quite rapidly after dosing has ceased.

The inhalation experiments demonstrate (Fig. 4) that the rat is capable of absorbing DNOC through the lungs, and that prolonged inhalation of such an aerosol may kill the animal.

In view of the report from Alberta (1947) a close watch was kept on the animals exposed to the aerosol. The rats were placed in the tank once a steady flow of the aerosol was established, that is about half an hour after switching on the Aerovap. During the first half hour of exposure the rats were moderately active, thereafter they became quies-

Table 3. *Accumulation of DNOC in blood of animals*

(D + 1, D + 2, etc. represent 1 and 2 days after dosing on D day. Where the found value of *t* is equal to or greater than the theoretical 5% level it is in heavy type. Significant individual slopes and differences are also in heavy type. The numerical values of the slopes are equivalent to $\mu\text{g. DNOC/g. blood/day}$. The value *t* in col. 6 is calculated on grouped values obtained on D + 1 and D + 2.)

Animal + sex	Wt. (g.)	Dose (daily) (mg./kg.)	DNOC concentrations in blood ($\mu\text{g./g.}$)			Individual slopes $\pm$ s.e. D + 3 onwards	Mean slope of group $\pm$ s.e.	Remarks
			D + 1	D + 2	<i>t</i>			
Rabbit F.	1595	8 $\times$ 25. Oral by stomach tube	2.8	2.8	1.44	+0.12 $\pm$ 0.16	+0.41 $\pm$ 0.25	Because there is no significant increase from D + 1 to D + 2 slopes calculated from D + 2 onwards
	1406		3.7	4.0		-0.13 $\pm$ 0.33		
	1616		10.0	8.4		+0.09 $\pm$ 0.37		
	1805		4.1	6.6		+0.72 $\pm$ 1.55		
	1605		2.7	3.6		+1.52 $\pm$ 0.10		
	1400		6.4	5.2		+0.11 $\pm$ 0.22		
Rats (hooded) F.	175	8 $\times$ 20. Oral as above	48.0	57.0	6.20	-3.80 $\pm$ 1.47	-1.53 $\pm$ 0.88	Died D + 3 Died D + 5
	165		47.0	63.0		—		
	184		52.0	58.0		—		
	175		37.0	47.5		-0.74 $\pm$ 1.24		
	169		35.0	52.5		-1.90 $\pm$ 1.09		
	163		41.0	50.0		+0.34 $\pm$ 0.95		
Rats (hooded) M.	232	8 $\times$ 10. Oral as above	10.0	23.0	13.24	+0.46 $\pm$ 0.71	-1.42 $\pm$ 0.32	—
	252		12.0	22.0		+1.37 $\pm$ 0.54		
	236		12.5	27.0		+2.70 $\pm$ 1.41		
	196		9.4	25.0		+1.76 $\pm$ 3.14		
	192		14.0	26.0		+0.81 $\pm$ 3.65		
	196		10.0	27.0		+1.40 $\pm$ 0.17		
Rats (hooded) M.	231	8 $\times$ 5. Oral as above	5.5	9.3	7.61	+1.41 $\pm$ 0.50	+1.37 $\pm$ 0.24	—
	253		6.9	12.5		+0.43 $\pm$ 0.61		
	212		5.8	12.5		+1.62 $\pm$ 0.53		
	206		4.3	12.5		+1.60 $\pm$ 0.48		
	240		5.5	14.0		+1.80 $\pm$ 0.60		
Rats (Albino) M.	225	14 $\times$ 10. Intraperi- toneal injection	17.2	25.0	5.20	-0.42 $\pm$ 0.87	-0.35 $\pm$ 0.23	—
	145		21.5	28.5		-0.26 $\pm$ 0.22		
	198		22.0	31.2		-0.67 $\pm$ 0.28		
	139		27.0	48.5		-1.17 $\pm$ 0.42		
	143		33.5	47.5		-0.12 $\pm$ 0.40		
Rats (Albino) M.	154	14 $\times$ 5. Intraperi- toneal injection	14.2	25.0	2.12	-0.58 $\pm$ 0.16	10.58 $\pm$ 0.24	—
	186		3.6	17.9		-0.36 $\pm$ 0.18		
	117		21.8	16.6		-0.42 $\pm$ 0.23		
	115		14.7	23.5		+0.18 $\pm$ 0.32		
	147		13.6	13.2		-0.70 $\pm$ 0.20		
F.	115		20.8	32.5		-1.60 $\pm$ 0.43		
	147		20.8	32.5		-1.60 $\pm$ 0.43		

cent, eating very little food and drinking little water. During the progress of the experiment the fur became yellow, but the rats showed no inclination to lick off any DNOC from themselves or from their fellows. Rats were removed at intervals and blood samples taken. On killing the animals at the end of the first experiment the lungs and alimentary canal were analysed for DNOC and gave values as follows ($\mu\text{g./g.}$). Lungs (drained free from blood): 16, 20, 31, 28. Alimentary canal and contents: 2.5, 3.1, 2.8, 2.2.

These figures suggest that most of the DNOC in the blood arises by inhalation of the aerosol since ingestion of any contaminated food and water would have resulted in higher DNOC values for the alimentary tract (see Table 1). It is also unlikely

that much DNOC had been absorbed through the skin since shaving of the hair after death revealed a white skin.

Some observations were made on the physiological effect of DNOC aerosols on rats (Table 5). Continued exposure to a DNOC aerosol causes increases in the blood DNOC, the respiration rate and the body temperature. At the end of 5 hr. the animals were removed from the aerosol and kept at room temperature. Twenty hours later four out of five blood DNOC values had decreased, but there was a general increase in the temperatures and respiration rates. This suggested that although the DNOC level had decreased there was some accumulation of its general metabolic effects.

Table 4. *Effect of daily doses of DNOC on weight of rats*

Dose (a)	Mean wt. $\pm$ s.e. (g.)			
	Saline control	5 mg./kg.	10 mg./kg.	25 mg./kg. (b)
At commencement of exp. (D day)	155 $\pm$ 1.82	160 $\pm$ 1.59	154 $\pm$ 2.26	156 $\pm$ 1.64
Immediately before first dose, D + 7	162 $\pm$ 2.06	163 $\pm$ 2.05	161 $\pm$ 3.16	163 $\pm$ 2.63
Difference, D and D + 7	+7.0	+3.0	+7.0	+7.0
Immediately before penultimate dose, D + 15	165 $\pm$ 1.93	170 $\pm$ 2.33	158 $\pm$ 2.69	155 $\pm$ 2.65
Difference, D + 7 and D + 15	+3.0	+7.0	-3.0	-8.0
At last day of experiment, D + 29	173 $\pm$ 3.18	177 $\pm$ 2.55	165 $\pm$ 2.65	177 $\pm$ 6.24
Difference, D and D + 29	+18.0	+17.0	+11.0	+21.0
<i>t</i> (D + 7 and D + 15)	(+) $\cdot$ 1.19	(+) $\cdot$ 1.32	(-) $\cdot$ 3.25 (d)	(c)

(a) All doses given by stomach tube. DNOC made up in 0.9% saline. Saline control started at same time as the other doses.

(b) Two rats died on D + 7 and one on D + 8. The mean values are taken on the remaining three rats.

(c) Since the sample size is only three, *t* test not applied.

(d) Significant.

Fig. 3. Effect of DNOC on weight of rats. Mean values of six rats per group. ----, Control, 0.9% saline; —, DNOC, 5 mg./kg.; - · - · -, DNOC, 10 mg./kg.; · · · · ·, DNOC, 25 mg./kg. (three rats died before dosing was completed).

Fig. 4. Effect of DNOC aerosol on rats: blood DNOC values after exposure at 25° to concentration of 0.1 mg./cu.m. ●—●, Four albino rats (not previously dosed); ○—○, six hooded rats previously given sixteen daily doses 5 mg./kg. DNOC by intraperitoneal injection: dots and circles show values for individual rats; lines show mean value.

Table 5. *Effect of DNOC aerosol on body temperature, respiration rate and blood levels of rats*

(The sign (+) or (-) indicates rise or fall over previous value.)

Hr.	Rat no.	...	1	2	3	4	5
Placed in DNOC aerosol, 100 mg./cu.m. for 4 hr. at 28-30°							
0	Temp. (° F.)		96	95.5	95.5	98.5	101.5
	Resp. rate		200	200	170	240	26
	Blood DNOC (μg./g.)		0	0	0	0	0
1	Temp. (° F.)		93.5 (-)	92.5 (-)	96 (-)	98.5 (-)	97 (-)
	Resp. rate		190 (-)	200	200 (+)	240	200 (-)
	Blood DNOC (μg./g.)		6.0 (+)	4.0 (+)	6.0 (+)	21.0 (+)	50.0 (+)
3	Temp. (° F.)		95 (+)	95 (+)	95	96	99
	Resp. rate		190	170 (-)	170 (-)	170 (-)	240 (+)
	Blood DNOC (μg./g.)		24.0 (+)	13.0 (+)	9.0 (+)	37.0 (+)	42.0 (-)
4	Temp. (° F.)		99.5 (+)	97 (+)	95	96	99
	Resp. rate		210 (+)	180 (+)	150 (-)	200 (+)	240
	Blood DNOC (μg./g.)		35.0 (+)	21.0 (+)	16.0 (+)	44.0 (+)	64.0 (+)
Removed and kept at room temperature overnight (20-22°)							
20	Temp. (° F.)		96 (+)	97 (+)	100 (+)	102 (+)	99
	Resp. rate		150 (-)	280 (+)	210 (+)	300 (+)	240
	Blood DNOC (μg./g.)		22.0 (-)	17.0 (-)	20.0 (+)	27.0 (-)	29.0 (-)
	All survived		—	—	—	—	—

The initial experiments on the application of DNOC to the skin of rabbits indicated that heat might cause an increase in absorption of DNOC if increases in blood DNOC were to be taken as evidence of this. These results are set out in Figs. 5 and 6. In order to check this point a larger experiment was carried out using two groups of rabbits

The effect of heat on the general metabolism of animals dosed with DNOC is very marked (Parker *et al.* 1951). The results (Table 6) show that by doubling the dose of DNOC (from 20 to 40 mg./kg.) the mortality of rats at 37-40° is increased from 6/12 to 12/12, whereas at the lower temperature (20-22°) the increase is only from 0/12 to 2/12.

Fig. 5. Skin absorption of DNOC by albino rabbits. 1% aqueous (w/v) DNOC (as Na salt) to give 1 mg./sq.cm. over 100 sq.cm. four rabbits: rabbit 1, x---x; rabbit 2, ●---●; rabbit 3, ○---○; rabbit 4, ■---■. All put in hot room at 37-40° after second application at times shown.

Fig. 6. Skin absorption of DNOC by Himalayan rabbits. 2% DNOC (w/v) (as Na salt) in 10% aqueous glycerol (w/v) to give concentration of 2 mg./sq.cm. over 50 sq.cm. Rabbit 5, x---x, and rabbit 6, ○---○, both put in hot room at 37-40° after second application. Rabbit 7, ●---●, kept at room temperature, 20-22°.

(Fig. 7) and Student's *t* test was applied to the grouped blood-DNOC slopes of those animals kept at 37-40 and 20-22°. In the first series of experiments albino and Himalayan rabbits were used and in the second chinchillas.

Although heat has this striking general metabolic effect it does not appear to alter the DNOC blood levels. Whether the dose is high, e.g. 40 mg./kg., or low, e.g. 5 mg./kg., the respective blood DNOC levels are unaltered by a rise in environmental

Fig. 7. Skin absorption of DNOC by twelve Chinchilla rabbits. 2% aqueous (w/v) DNOC (as Na salt) to give 2 mg./sq.cm. over 50 sq.cm. Group A, $\bigcirc$ — $\bigcirc$ (broken line mean value); group B, $\bullet$ — $\bullet$ (continuous line mean value). Slopes from 8.0 to 12.0 hr. A: -2.05 , $+2.68$, -1.50 , -1.48 , -1.65 , -3.08 ; B: -2.42 , -3.49 , -2.35 , -1.19 , -2.26 , -2.70 . $d(\bar{X}_A - \bar{X}_B) = 1.79$, s.e. (d) = 0.96 not significant.

Table 6. *Effect of temperature on mortality of rats given DNOC by stomach tube*

Dose (mg./kg.)	Temperature (°)	No. of rats used	No. of rats alive					Mortality	
			Time after dosing (hr.)					Number	%
			2	4	6	8	24		
5	20-22	12	12	12	12	12	12	0/12	0
	37-40	12	12	12	12	12	12	0/12	0
10	20-22	12	12	12	12	12	12	0/12	0
	37-40	12	12	12	12	12	12	0/12	0
20	20-22	12	12	12	12	12	12	0/12	0
	37-40	12	12	12	11	6	6	6/12	50
30	20-22	12	12	12	12	12	12	0/12	0
	37-40	12	12	10	4	2	2	10/12	83
40	20-22	12	11	11	10	10	10	2/12	16
	37-40	12	10	4	0	0	0	12/12	100
50	20-22	12	12	10	9	9	8	4/12	33
	37-40	12	6	1	0	0	0	12/12	100
60	20-22	10	8	0	0	0	0	0/10	100
	37-40	10	3	0	0	0	0	0/10	100

temperature (see Table 7). Although this communication deals essentially with the blood DNOC levels of animals given daily doses, a few experiments have been included to show the effects of repeated 2 hr. doses at different levels on rats and rabbits (Fig. 8; cf. *Brit. med. J.*, 1951). After six 2 hr. doses of 20 mg./kg. both the rat and the rabbit died. The other animals survived.

DISCUSSION

Evidence is presented that DNOC can be absorbed very readily from the alimentary canal, through the lungs and through the skin, and that repeated daily

doses of DNOC appear to undergo limited accumulation in the rat but insignificant accumulation in the rabbit.

Absorption from the alimentary canal

Although there is some evidence that negative slopes may result from large oral doses given to the rat, those from smaller doses are positive, and their mean is significant. It may be argued from this that there is a tendency to reach an intermediate and 'stabilized' blood level greater than those which are attained after eight small daily doses, but less than those resulting from the large doses.

Table 7. *Effect of heat on blood DNOC levels of rats following one dose of 40 mg./kg. given by stomach tube*
(All levels in $\mu\text{g./g.}$ Figures in brackets denote number of animals per group.)

Time sampled (hr. after dose)	Group A. Kept at 20–22°. Twelve hooded rats. Weights 179–189 g. (mean wt. 183 g.)			
	Living		Immediately after death*	
	Range	Mean $\pm$ s.e.	Range	Mean $\pm$ s.e.
2	90.0–110.0 (11)	100.3 $\pm$ 1.98	97.0 (1)	—
4	89.0–123.0	105.5 $\pm$ 2.85	None	—
6	93.0–108.0 (10)†	97.4 $\pm$ 1.53	91.0 (1)	—
Group B. Kept at 37–40°. Twelve hooded rats. Weights 174–195 g. (mean wt. 194 g.)				
2	83.2–111.0 (8)	93.9 $\pm$ 4.31	69.4–113.0 (4)	93.5 $\pm$ 9.65
4	94.5–113.0 (3)	100.8 $\pm$ 6.09	80.0–104.0 (5)	88.0 $\pm$ 4.57
6	None	—	89.5–104.0 (3)	96.8 $\pm$ 4.18

* Blood obtained within 5–10 min. of death. In many cases the heart was still fluttering although breathing had ceased and rigor had set in.
† One blood sample lost.

The two methods used for the administration of DNOC give different results. Absorption of DNOC

without the delaying barrier of the gut wall. This could account for the rapid appearance of the high blood DNOC levels to which reference has already been made. It is less easy to account for the negative slopes resulting from repeated intraperitoneal injections of DNOC. Some light may be shed on this aspect by an examination of the method used for the presentation of the results. Perusal of the daily blood DNOC levels of the individual animals indicated that they appeared to show very little regular variation from day to day. This led to the assumption that the calculations of the slopes of the blood DNOC values onwards from the second and third days following dosing might be expressed in a linear manner. This is probably justified in the present study. While it is conceivable that continued oral doses of 5 and 10 mg./kg. might accumulate at the rate of 1.3 $\mu\text{g./g./day}$ until a higher level is reached, yet it is less conceivable that zero blood DNOC levels will result if the process of daily dosing is extended for periods far in excess of those recorded in the present investigations. The fact that the mean slopes of the three groups concerned are not significant suggests that extended slopes would be much flatter than the significant individual values appear to suggest.

Absorption through the lungs

The results of the inhalation experiments suggest that this method of absorption is likely to be particularly dangerous. Two general facts support this hypothesis. First, small particles or droplets containing DNOC which are less than, say, 0.5 μ , will

Fig. 8. Accumulation of DNOC in rat and rabbit following six 2 hr. doses of varying levels. Rats, $\bigcirc$ — $\bigcirc$; rabbits, $\bullet$ — $\bullet$. Blood DNOC levels resulting from single doses of DNOC of same magnitude given at commencement of test: rat, $\triangle$; rabbit, $\blacktriangle$.

from the lumen of the alimentary canal must include its passage through the canal wall before it can enter the hepatic portal system.
DNOC placed in the peritoneal cavity by injection also passes into the hepatic portal system but

probably be carried directly in the inspired air to the lung surface. The DNOC will then be absorbed and rapidly and efficiently distributed throughout the body without the intermediate filter of the liver. In other words, it is likely that any given quantity of DNOC actually in the lung will be more rapidly absorbed and more efficiently distributed than an identical dose absorbed through the gut or through the skin. Secondly, the solubility of DNOC and its simpler derivatives in water will permit the ultimate absorption of the large particles which may fall on to any part of the mucosa of the respiratory or alimentary tract. Although DNOC itself is not very soluble in water (0.02%), the more widely used sodium and ammonium salts are much more soluble (about 7.5 and 0.9% respectively) and are therefore more dangerous.

Absorption through the skin

The problem of assessing the significance of the blood DNOC levels following its absorption through the skin is complicated by many variable factors. These include species of animal, the nature of the substance absorbed and the environmental temperature. The latter appeared to be very important from the practical point of view.

Pilot experiments on albino and Himalayan rabbits suggested that heat had a definite effect in increasing the blood DNOC level, probably by increasing skin absorption, and that the increase was sometimes delayed (Figs. 6 and 7). On a larger number of chinchilla rabbits a high environmental temperature did not appear to cause any increase in the blood DNOC values. In spite of the contradictory nature of the evidence in this respect three definite conclusions can be drawn from the experiments. First, DNOC can be absorbed through the skin of the rabbit. Secondly, repeated doses (within 6 hr.) cause an increase in the blood DNOC level. Thirdly, the skin can act as a reservoir for DNOC. This is illustrated by the fact that 48 hr. after application of DNOC to the skin of chinchillas the blood DNOC levels ranged from 2.4 to 7.9 $\mu\text{g./g.}$ These levels are obviously significant even if they are small, particularly when it is considered that single large doses of DNOC administered by any of the other methods may be completely eliminated within 24 hr.

Effect of heat and critical level of blood DNOC

Increased environmental temperature has a marked effect on the metabolism of an animal poisoned with DNOC, but no effect on the blood DNOC levels, unless skin absorption is increased. It seems, therefore, that an increase in environmental temperature builds up the increased metabolic effect of DNOC, but does not initiate or stimulate any reactions affecting the linkage of DNOC to any intracellular or extracellular substances.

A further deduction that can be made from the experiments described is that the blood DNOC levels of the rat following any method of administration and absorption are unlikely to exceed about 100 $\mu\text{g./g.}$ Thus an intraperitoneal injection of 20 mg./kg. (Table 2) will result in the rapid attainment of a level of 100 $\mu\text{g./g.}$ and this falls off to about 60% of its value in 5 hr. (Table 2). On the other hand, a single dose of 40 mg./kg. given by stomach tube results in the same level (100 $\mu\text{g./g.}$) which is maintained for at least 6 hr. The survivors and victims of this dose all have approximately the same blood DNOC levels whether they have been exposed to high (37–40°) or moderate (20–22°) temperatures. Prolonged exposure to an aerosol of DNOC gives similar results, and blood DNOC levels of those animals previously dosed with DNOC appear to increase less rapidly once levels of 80–90 $\mu\text{g./g.}$ are reached. The blood DNOC value of one animal reached an unusually high peak value of 130 $\mu\text{g./g.}$ Two main deductions may be drawn from these various observations. First, the blood of the rat is saturated with respect to DNOC when the blood level is about $100 \pm 10 \mu\text{g./g.}$ Secondly, that a level of $100 \pm 10 \mu\text{g./g.}$ is critical for the rat in so far that further absorption of DNOC, or exposure to an increased environmental temperature will result in severe symptoms or in the death of the animal. On the other hand, neither of these factors will cause any appreciable difference in this critical blood DNOC level.

Comparison between man, the rat and the rabbit

The experiments indicate that the rabbit has more efficient mechanisms for eliminating DNOC than has the rat, hence it cannot readily accumulate daily doses of DNOC. The results of the trials on human volunteers (Harvey *et al.* 1951) show that man can accumulate doses of about 1 mg./kg./day and therefore indicate that man is much less efficient in dealing with DNOC than either the rat or the rabbit.

On the other hand, the statement 'DNOC is only cumulative when doses follow one another in rapid succession in periods of hours and not days' (*Brit. med. J.* 1951) is misleading. For, although this can apply to the rat and the rabbit (*cf.* Fig. 8), it does not apply to man.

At least one interpretation of this statement is that a man handling DNOC would have to absorb several daily doses of DNOC before he could accumulate it; obviously this implies that he possesses more efficient mechanisms for eliminating DNOC than is suggested by the results of the human trials and the examination of actual DNOC casualties (King & Harvey, 1952, 1953). The difference in quantitative response towards DNOC as exhibited by the rat and the rabbit is further illustrated by

Fig. 8. Six identical 2 hr. doses cause the rat and the rabbit to build up DNOC concentrations at different rates; whereas the rat attains a maximum critical value of about 108 $\mu\text{g./g.}$ before death, on 20 mg./kg. the rabbit does not exceed a level of more than 67 $\mu\text{g./g.}$ on the same dose.

Further evidence of species difference in quantitative response towards DNOC will be published in other communications (King & Harvey, 1952, 1953).

The practical outcome for the DNOC spray operator and worker would seem to be that the most dangerous paths of absorption of DNOC are through the lungs and through the alimentary canal. A less dangerous route is through the skin, but this can obviously act as a reservoir for a small but more continuous supply to the blood. Harvey *et al.* (1951), Bidstrup *et al.* (1952) and Harvey (1952) have suggested that for man a blood DNOC level of 20 $\mu\text{g./g.}$ is dangerous in that toxic symptoms are then likely to occur. The animal experiments show that heat will enhance the toxic effect of any dose of DNOC but will not affect the blood DNOC level. Therefore, any blood DNOC level of a man working with DNOC that is accepted as an indication of early toxic symptoms should be treated with more care if the weather is hot than if it is cold.

SUMMARY

1. 4:6-Dinitro-*o*-cresol (DNOC) can be absorbed into the blood of rats and rabbits by ingestion, by inhalation and through the skin.

2. The rat can maintain significant blood DNOC levels following repeated daily doses of 5–20 mg./kg. Definite accumulation of DNOC in the blood occurs with two daily doses, but thereafter repeated daily doses cause very slight increases or decreases which are of doubtful significance.

3. An apparatus is described for producing a DNOC aerosol.

4. The rabbit can absorb DNOC through the skin. Repetition of the application caused increases in blood DNOC levels.

5. At levels of about 100 $\mu\text{g./g.}$ of blood rats may die either as a result of further doses of DNOC, or as the result of an increase in the environmental temperature.

6. Increases in environmental temperatures cause the mortality of rats dosed with DNOC to increase, but they do not cause any significant changes in blood DNOC levels.

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