III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Dinitro-ortho-cresol, $C_7H_6N_2O_5$, is a yellow crystalline solid derived from o-cresol. There are six DNOC isomers, but the 4,6-dinitro isomer (see structure in Figure XIII-1) is the most commercially important. DNOC is produced either by sulfonation of o-cresol followed by treatment with nitric acid or by treatment of o-cresol in glacial acetic acid with nitric acid at low temperatures [1]. Some important chemical and physical properties of DNOC are shown in Table XIII-1 [2-4].

DNOC was introduced in 1892, in its potassium salt form, as the active ingredient of the pesticide "Antinonnin," used for controlling the nun moth [5]. DNOC is still primarily an agricultural chemical, although it has had limited use in the dyestuff industry and for other minor, miscellaneous industrial purposes. Currently, it is used primarily as a blossom-thinning agent for fruit trees and as a fungicide, insecticide, and miticide applied to fruit trees during the dormant season. Its use for these purposes is confined mainly to the Pacific Northwest.

DNOC has been used less in recent years because it is highly toxic to plants in the growing stage and nonselectively kills both desirable and undesirable vegetation. The Environmental Protection Agency has no record of DNOC being currently manufactured in the United States for use as an agricultural chemical. Imports of DNOC have also decreased in recent years; from 217,899 pounds in 1972 to 146,621 pounds in 1973 and then to

30,442 pounds in 1976. Currently, only one company in the United States formulates the sodium salt of DNOC, which is marketed under the trade name Elgetol. They obtain DNOC by importing the product from Japan. According to a spokesman for this company, Elgetol is formulated once a year on a customer-request basis, and only 3-10 workers, at most, are potentially exposed during its production. Another company announced in 1976 that it would discontinue its small-scale production and formulation of DNOC. Pesticide sprayers are therefore the major group with potential occupational exposure to DNOC. In addition to the DNOC sold for agricultural use, a few chemical distributors sell small amounts of technical grade DNOC for laboratory purposes.

NIOSH estimates that 3,000 workers in the United States are potentially exposed to DNOC.

Historical Reports

First introduced in 1892 for use against the nun moth [5], DNOC began to draw attention in 1925 for its utility in agriculture and horticulture [6]. Since then, several deaths attributed to exposure to DNOC have occurred in various countries. In addition to its use in agriculture, DNOC was introduced in 1933 as an alternative to dinitrophenol (DNP) in the treatment of obesity [7]. However, high doses caused cataracts, blindness, and death in many people, and as a result DNOC was used for this purpose for only a short time.

Dodds and Pope [8], in 1933, observed that DNOC was three times as potent as DNP in elevating the oxygen consumption rate in guinea pigs.

They therefore thought that less DNOC than DNP could be used to increase the basal metabolic rate (BMR) and produce weight loss. In an attempt to find a safe dose for humans, Dodds and Robertson [9] gave DNOC to a number of healthy young adult volunteers, who were of average weight or overweight. A daily oral dose of 3 mg/kg of body weight produced toxic effects by the 3rd day, when the BMR had increased by an average of 50%. By the 4th day, the BMR had increased as much as 100%. The volunteers experienced profuse sweating, lethargy, headache, loss of appetite, and greenish-yellow pigmentation of the conjunctiva. Treatment with DNOC was then stopped, whereupon the signs and symptoms disappeared. In a second experiment, one 3 mg/kg oral dose of DNOC produced a rapid rise in the BMR to 20-30% higher than the preexposure level within 24 hours. The rate returned to normal 4-5 days later. It was found in a third experiment that 50-100 mg/day (0.5-1.0 mg/kg of body weight) was needed to maintain a BMR 30-50% greater than normal, an increase which the authors reported would not be accompanied by toxic signs or symptoms.

Ibrahim et al [10] reported in 1934 adverse effects in people taking DNOC to lose weight. The authors studied 15 people, 8 men and 7 women, aged 17-38 years, who had taken doses of 50 or 100 mg/day for an average of 7 weeks. After the patients had taken DNOC for a few days, they all developed signs and symptoms of DNOC intoxication, including excessive sweating, thirst, fatigue, decreased appetite, and elevated BMR. Their conjunctivae became greenish yellow.

Other investigators [11,12] also observed toxic effects in persons taking DNOC for weight reduction purposes. In 1936, Plotz [11] described effects similar to those reported by Ibrahim et al [10] in three persons

who had each taken between 0.35 and 1.5 mg/kg/day of DNOC for up to 9 weeks. In 1937, Quick [12] reported the development of cataracts and blindness in a woman who had taken DNOC for 3 years and he noted that a number of other people had developed cataracts after taking DNOC.

Hunter [7] wrote that, by 1937, many poisonings and some deaths had resulted from the use of DNOC for weight reduction purposes. He stated that at least three deaths in Great Britain had been caused by overdoses of DNOC and that cataracts and blindness had developed in some patients months after they had stopped taking DNOC. Although he noted that less than 1% of those who were treated with DNOC developed complications, he considered the difficulty of setting a safe dose for each individual to be the reason that its use as an aid to weight loss was discontinued.

In many countries, deaths from DNOC exposure occurred among workers in the plants where it was manufactured [7]. DNOC dust was apparently the most dangerous form because it was readily inhaled and quickly produced effects. Workers experienced excessive sweating, thirst, a feeling of weakness, and loss of weight. During the summer of 1943, 14 poisonings were reported in a factory in Great Britain where DNOC dust was prepared for use against locusts [7]. After local exhaust ventilation and periodic medical examinations were introduced in the factory, only one mild case was reported.

Bidstrup and Payne [6] noted in 1951 that environmental temperature influenced the severity of intoxication in workers exposed to DNOC. They observed that all the reported fatalities attributed to DNOC poisoning in Great Britain between 1946 and 1950 occurred during what the authors considered to be "unusually hot" weather (56-86 F).

Effects on Humans

Other than those reports dealing with its use as a weight-reducing drug [7,10-12], only a few were found in which authors described the effects of DNOC in nonoccupational exposure situations. The relationship between blood DNOC levels and intoxication has been investigated [13], and effects of DNOC ingestion [13,14] and skin contact [13,15,16] have been observed.

Harvey et al [13], in 1951, described the effects of DNOC taken orally by five male volunteers. Each man was given capsules containing 75 mg of pure DNOC daily for 5 consecutive days, amounting to a dose of 0.92-1.27 mg/kg/day. The concentration of DNOC in the blood was measured 30 minutes before and 1, 2, 4, and 6 hours after each dose was taken and then at various intervals up until 40 days later.

The concentration of DNOC in the blood increased for the first 3-4 days and reached concentrations of 15-20 μg/g [13]. After these concentrations (15-20 μg/g) had been attained, additional doses appeared to cause temporary high blood concentrations which were associated with symptoms. The man receiving the largest daily dose (1.27 mg/kg) showed a peak concentration of 40 μg/g after the fifth dose. The man who had been given 0.92 mg/kg/day received additional DNOC on the 6th and 7th days, which caused the blood DNOC level to rise to 40 and then to 48 μg/g on these 2 days. In both of these men, the high blood DNOC levels were associated with symptoms of poisoning, including lassitude, headache, and malaise. Conjunctival staining was seen by the 4th day in all five volunteers. The concentration of DNOC in the blood was temporarily increased in three of the men when they performed 30 minutes of exercise on

the 8th and 9th days. A 2% aqueous solution of DNOC applied to the skin of three men on day 12 also caused a slight rise in the blood DNOC concentration. DNOC was slowly eliminated from the body. It was still detected in the blood (1 μg/g in each of four subjects) 40 days after the final dose was given.

In 1952, Bidstrup et al [17] discussed additional findings that related to their original study [13]. Regarding the human volunteers given DNOC orally, the authors noted that temporary high blood DNOC concentrations were observed only when the blood was sampled less than 8 hours after the last exposure. This phenomenon occurs because DNOC binds with albumin in the blood [13] and is therefore not rapidly distributed to the body tissues. Apparently, significant distribution occurs 8 hours after exposure to DNOC, and blood samples taken after this time lapse show blood DNOC levels that correlate better with observed signs and symptoms of toxicity. The owner of a firm of contract sprayers informed them that one of the earliest signs of DNOC exposure was a "fitter than usual" feeling in the workers [17]. He gave the example of a man who, because he was feeling well, protested being transferred to another job. As a result of this observation, the authors [17] reevaluated their previous observations [13] and found that, on the 3rd or 4th day of the experiment, all of the volunteers had experienced an exaggerated feeling of well-being. At this time their blood DNOC levels were about 20 μg/g. Bidstrup et al [17] commented that the importance of this finding had not been recognized at the time of the experiment, and it was not mentioned in the earlier report [13].

Sovljanski et al [14], in 1971, reported two suicides by ingestion of known amounts of DNOC. One person had swallowed 50 g of DNOC, and the other, 140 g. Analysis of tissue samples revealed DNOC in the stomach, intestines, liver, kidneys, heart, and brain, with the stomach containing the greatest amount. Blood DNOC levels were not reported.

Observations have been recorded on the effects of DNOC after intentional or unintentional dermal contact. Ambrose [15] investigated the effects of cutaneous application of DNOC to humans. A 2% aqueous solution of the sodium salt of DNOC was applied daily for 30 days to the shaved armpits and forearms of two volunteers. Neither local skin irritation nor systemic effects were observed.

In 1974, Buchinskii [16] reported the death of a 4-year-old boy after DNOC was applied to his skin. A rash had been treated with 50 g of an ointment to which DNOC was added by mistake. The child began vomiting 1 hour later and developed a headache. When he was hospitalized 2 hours after the ointment was applied, he was confused, and his skin, sclera, and what was translated as visible mucosa were stained yellow. His pulse rate was 96 beats/minute, and his respiratory rate was 45/minute. Within half an hour, he was unconscious. Tachycardia developed, and moist rales were detected in the lungs. The boy had convulsions, and death followed 3.5 hours after the ointment was applied. An autopsy showed diffuse petechial hemorrhages in the intestinal mucosa and brain and pulmonary edema. Microscopic examination of several tissues revealed capillary congestion in the brain, liver, lungs, intestinal walls, myocardium, and kidneys. An unspecified amount of DNOC was detected in the blood. Analysis of the ointment showed that it contained 25% DNOC.

Several reports of injuries and deaths of workers exposed to DNOC have been noted in the literature [14,17-25]. Most of these occurred in agricultural sprayers, although some involved manufacturing workers. The signs and symptoms of intoxication were primarily related to the ability of DNOC to increase the metabolic rate. These included profuse sweating, thirst, a feeling of great heat, headache, fatigue, and an increased BMR. In addition, DNOC affected the nervous systems of a number of people, producing numbness in the limbs.

Studies of workers who were exposed to DNOC [17,19-23,25-27] have also examined the fate of DNOC in the blood and the association between blood DNOC levels and the severity of intoxication.

A case of industrial poisoning by DNOC occurred in the United States in a man involved in its manufacture [18]. The episode occurred in the early part of 1943, but the duration of exposure was not stated. The man was hospitalized with signs and symptoms that included a temperature of 102 F, a BMR greater than 400%, rapid pulse and respiration, profuse sweating, shortness of breath, and a cough. The man's palms and soles were stained yellow and it was reported that he had recently lost 20 pounds. He was treated successfully and recovered fully. It was determined that the level of airborne DNOC dust in the workplace was 4.7 mg/cu m. The methods used for sampling and analysis of DNOC were not reported.

In 1952, Bidstrup et al [17] published the details of a survey conducted to determine whether there was a correlation between blood DNOC levels and the onset of toxic symptoms. They collected blood samples from 195 individuals: 23 process workers involved in the manufacture of DNOC for 6 weeks to 5 years, 39 men who used DNOC as a winter wash spray on

fruit trees, 8 of whom had been spraying for more than 50 days, and 133 men who sprayed DNOC on cereal crops for 6 weeks during the summer. Of the cereal crop sprayers, 45 had a blood DNOC level above 10 μg/g, while only 1 process worker and none of the winter wash sprayers had blood DNOC levels that high.

Bidstrup et al [17] suggested that the process workers had lower blood DNOC levels than cereal crop sprayers because there was greater use of protective measures (not specified) in the factory. Only small quantities of DNOC were believed to have entered the blood through the skin. The authors cited the case of one man who had been involved in the manufacture of DNOC for 5 years. His hands, face, and hair were stained bright yellow, but the concentration of DNOC in his blood was only 7.3 μg/g. However, since no correlation has been established between the quantity of DNOC to which one is exposed and the degree of skin staining, the amount of DNOC with which the man came into contact cannot be estimated.

Winter wash sprayers also had less risk of DNOC intoxication than did cereal crop sprayers [17]. A much weaker solution of DNOC was used during the cold weather, and the method of spraying in winter produced larger droplets, which were less likely to remain airborne.

Of the 133 cereal crop sprayers, 20 had blood DNOC levels of 10-20 μg/g, 16 had 20-30 μg/g, 5 had 30-40 μg/g, and 4 had more than 40 μg/g [17]. The four workers with the highest blood levels of DNOC experienced acute poisoning, and one of these, who had a blood DNOC level of 75 μg/g, died.

Good correlation was found between blood DNOC levels and the development of symptoms in one worker who became seriously ill, as described by Pollard and Filbee [19] in 1951. The 27-year-old man was a member of a spraying team that had been applying DNOC to fruit trees for 5 weeks during May and June 1951 and was primarily responsible for mixing the DNOC solution and refilling the sprayer tanks. He had sprayed for a total of less than 3 hours and later said that he had worn the "regulation" personal protective equipment provided, although he had seldom used a face mask. He developed symptoms of poisoning, including headache and general lassitude, and was admitted to a hospital about 52 hours after he was last exposed to DNOC. His hair, sclera, and skin, especially that on his face, hands, and feet, were stained yellow. Vital signs and body temperature were measured, and biochemical investigations, including blood tests, urinalysis, and measurements of blood and urinary DNOC levels and the BMR, were made several times during the patient's 1-month stay in the hospital. DNOC was measured in the blood and urine almost daily.

On the 1st day of hospitalization, the man's body temperature was 102 F, and his pulse, respiration, and blood pressure were 100/minute, 25/minute, and 115/70, respectively [19]. Blood analysis on the 2nd day showed a hemoglobin concentration that was 80% of normal, normal total white and red cell counts, a low neutrophil count (38%), a high lymphocyte count (56%), and a high urea concentration (60 mg/100 ml). The patient's BMR was 275% of normal on the 3rd day and was still as high as 180% the 2nd week after exposure. By the end of the month, neutrophil and lymphocyte counts and urea level were close to normal. The DNOC concentration in the blood was 60 μg/g on the 1st day and fell slowly to 4 μg/g by the end of 1

month. The authors noted that the decreasing blood DNOC level corresponded roughly with the improvement in the patient's clinical condition and with a decrease in his BMR. Urinary urea was high (3.6-4.3 g/100 ml) on the first 3 days of hospitalization, the only days for which this parameter was reported. The daily urine volume was low (400-900 cc) for the first few days but then returned to normal (2,000 cc). DNOC was detected in the urine during the entire month. The excretion rate ranged from 9.5 mg/day on the 2nd day to a high of 22 mg/day on the 4th day, and was 5 mg/day a month after the exposure. An electrocardiogram taken at an unspecified time was normal. The patient's body temperature returned to normal by the 5th day, and his condition was already greatly improved. The authors noted that the most striking findings in the patient were high values for BMR, nitrogen excretion, and blood urea.

Varnai and Kote [20] reported that, in Hungary in 1967, 47 women from a crew of 81 people required hospitalization after working in an onion field sprayed with an aluminum salt of DNOC on the previous day. The workers had been instructed not to eat the onions and to wash their hands before eating, but no protective clothing was supplied. Work began in the morning, and, according to the authors, the first signs of poisoning were evident by 4:00 pm. No specific signs were reported. Work stopped at 6:00 pm, and the ill women were admitted to a hospital. They ranged in age from 15 to 44 years, but most were under 20. Three of the women were pregnant; one was in the 2nd month, one was in the 6th month, and one, who was in the 9th month, gave birth 3 days after the exposure episode. The authors characterized the extent of poisoning as mild, moderate, or severe on the basis of blood levels of DNOC, clinical symptoms, and the results of other

laboratory tests. Specific data from the laboratory tests were not reported. The DNOC concentration in the blood was measured in 45 women, although when the measurements were taken was not specified.

Of the 47 cases, 32 were described as mild, 12 as moderate, and 3 as severe [20]. Effects included liver and kidney damage, loss of weight, unconsciousness, visual disturbance, hemorrhaging, and fever. DNOC blood levels were associated with the severity of intoxication. In the patients considered to be moderately or severely poisoned, blood DNOC levels ranged from 20-55 μg/ml. These women were hospitalized for more than 8 days; one remained for 53 days. In contrast, the women who were mildly affected were released from the hospital by the 8th day. The DNOC levels in their blood were lower and ranged from 7 to 37 μg/ml. When a statistical analysis of the blood DNOC levels was done, it was found that the average blood DNOC level in workers who developed toxic effects (32.5 μg/ml) was significantly greater ($P<0.05$) than in those who exhibited no effects (26.1 μg/ml).

The authors [20] believed that DNOC induced labor in the woman who gave birth to a full-term healthy child 3 days after the exposure, but they gave no evidence to substantiate this. They also reported that the other two pregnant women gave birth to healthy children. Six weeks after the exposure, all of the patients were reexamined and declared healthy. It is assumed this included the woman who was still in the hospital, although no specific mention was made of her condition. Even though the women were declared to be in good health, many complained that they suffered from headaches when they worked in the sun.

In 1960, Van Noort et al [21] recounted five cases of DNOC poisoning in the Netherlands, one having a fatal outcome, that occurred in May and

June of 1954 and 1955. The victims were all men between the ages of 25 and 40 who had been spraying DNOC for a period of up to 4 months. The composition of the sprayed material was not specified. The men were all hospitalized and either had experienced or were still experiencing signs and symptoms of toxicity including profuse sweating, feelings of great discomfort from the heat, labored breathing, restlessness, fatigue, and thirst. It was noticed that their hair, skin (especially of the hands), sclera, and nails were stained yellow. The DNOC level in the serum varied from patient to patient. It was 200, 60, and less than 5 μg/ml in three men on the 1st day of hospitalization, and a fourth man had 10 μg/ml a month after he entered the hospital. The man who had a serum DNOC level of 5 μg/ml was not hospitalized until 3 weeks after exposure had ended. These four recovered from the poisoning, but the fifth man, who the authors stated had inhaled a large quantity of DNOC the day before he was hospitalized, went into a coma and died. His rectal temperature 30 minutes after death was 44.5 C, and the serum DNOC level at this time was 1,000 μg/ml.

Markicevic et al [22], in 1972, discussed the results of an examination of 27 workers exposed to DNOC while they were manufacturing a DNOC paste in Yugoslavia. The workers, all men aged 22-48 years (average 31 years), had been exposed to DNOC for 5-30 days prior to the examinations. It could not be ascertained whether exposure to DNOC was by inhalation, skin contact, or both. Eight men were examined between January and March 1968, 11 were examined in August 1968, and 8 others were examined in both the winter and the summer. Serum and urinary DNOC levels, BMR, erythrocyte sedimentation rate, pulse rate, blood pressure, and respiration

rate were measured. The DNOC concentrations in the serum and urine were measured 24 hours after DNOC was last handled. The physical appearance of the workers and their symptoms were also recorded.

The examination results indicated that 11 workers had no signs or symptoms of poisoning [22]. Sixteen others had yellow staining of the hair, nails, hands, and forearms. Two of the 16 workers had increased BMR's (+34% and +30%), while 2 others had increases that the authors attributed to the patients' lack of cooperation in taking the measurements. An increased BMR (+48%) was measured in another worker several weeks before he first was examined by Markicevic et al [22], when his BMR was +19%. It was +2% 11 days later.

Pulse, respiration, and erythrocyte sedimentation rates in the 27 workers were within normal ranges [22]. The serum DNOC levels ranged from 1.0 to 8.73 μg/ml and the concentration of DNOC in the urine ranged from nondetectable to 4.2 μg/ml. A statistical analysis of serum DNOC levels and skin staining, which was categorized as normal, yellow, or strongly yellow, showed there was a significant correlation ($P<0.05$) between serum levels and the degree of staining. In addition to yellow coloration of certain body tissues in 16 men, 1 of them experienced profuse sweating, nervousness, palpitations of the heart, and weight loss (6 kg in 1 month), 1 worker sweated excessively and complained of thirst, 1 had frequent diarrhea, and 1 had a blood pressure of 180/105. One of the workers whose skin was unstained also complained of sweating and had reddened conjunctivae and inflammation of the mucous membranes of the throat.

Burkatskaya [26] analyzed the blood of 20 Soviet workers (sex not specified) who prepared a 1% solution of DNOC for spraying fruit trees.

The length of exposure was not stated. At the time of spraying, the air temperature was 8-16.2 C and the relative humidity was 37-70%. Air samples were taken from the breathing zones of workers preparing the DNOC solution and loading it into sprayers. The methods used for sampling and analyzing DNOC were not described. Burkatskaya reported that the average airborne DNOC concentration was 0.0036 mg/liter (3.6 mg/cu m). In 13 of the workers examined, the DNOC concentration in the blood was 3-5 mg% (30-50 μg/ml), and it ranged from traces to 2 mg% (20 μg/ml) in the other 7. No signs or symptoms of poisoning were described.

Several authors [14,23,24] have described effects of DNOC exposure on the peripheral and central nervous systems, in addition to metabolic effects.

Stott [23], in 1956, detailed the effects on two men of DNOC absorbed through the skin. One, aged 47, had worked for 2 months cleaning the aircraft booms used to spray a 20% solution of DNOC in oil. He wore no protective equipment. The other, aged 24, cleaned and serviced aircraft spray systems in which a 20% solution of DNOC in oil was used. He worked for 10 days in the field and 1 week at the home base. The only protective clothing he wore was overalls. Each man said that he washed before eating and did not smoke. Since neither man worked near the actual spraying operation and both denied blowing into the spray jets to clean them, Stott concluded that the major route of exposure was skin contact.

The older man began to notice symptoms about 1 month after his first contact with DNOC [23]. Initially, he felt a prickling sensation on the back of his hands and fingers, which later spread to the legs. He also was sweating excessively on the lower parts of his arms and legs. When he

entered the hospital 3 days after his exposure to DNOC ended, he mentioned that he had been unusually thirsty during the exposure period but that his appetite was unaffected. A medical examination was performed, which included testing for sensation in his limbs, determining DNOC levels in the serum, and measuring his BMR. His palms and soles were stained yellow, his body temperature was 37.3 C, and his pulse rate was 80/minute. He was sweating heavily on the lower parts of his arms and legs, on the backs of his hands, and on his feet, and he felt no sensation from pinprick or cotton wool on the top of his fingers and toes. Depression of the right knee-jerk reflex was the only other indication of abnormality in the nervous system. The serum DNOC level was 7.6 μg/ml 1 week after the last exposure, and the BMR was +6% after 2 weeks. Values for these two indices immediately after the exposure were not reported. The man's condition improved rapidly, and he showed no signs or symptoms of intoxication after 9 days in the hospital.

The second man came to the hospital immediately after his exposure to DNOC ended [23]. He complained that he had had a tingling sensation on the backs of his fingers for the past 4 days and that his legs were numb at night. The man thought that he had lost some weight but did not experience excessive sweating or thirst. A medical examination was performed to test for neurologic disorders. No loss of sensation to pinprick or cotton wool on his hands or feet was detected, and no other effects on the nervous system were evident. His hands were stained yellow, and there was a petechial rash over his left shoulder. His serum DNOC levels measured 1 week after exposure began and on the last day of exposure were 16.8 μg/ml and 11.5 μg/ml, respectively. The patient was free of all symptoms within

1 week after exposure had ended. Stott considered the peripheral neuritis observed in both patients to be the result of local action by DNOC where it contacted the skin rather than a generalized systemic effect of DNOC after it had been absorbed into the bloodstream.

Buzzo and Guatelli [24] published a report in 1949 of two deaths in Argentina caused by DNOC exposure. Three brothers, aged 17, 21, and 21 years, had sprayed a powdered material that contained 10% DNOC for 2 consecutive days when there was a strong wind, an ambient temperature of 38 C, and a relative humidity of 70%. The personal hygiene of the men, as reported by the surviving brother, was poor. They did not change their underwear or wash before continuing work on the 2nd day. In the late afternoon of the 2nd day, two of the men complained of discomfort, thirst, and excessive sweating, and one of them was unable to walk without assistance. The three men were admitted to a nearby clinic. They had previously been in good health, and they had no history of smoking or drinking.

One of the 21-year-olds and the 17-year-old died shortly after they entered the clinic [24]. Each had exhibited identical signs and symptoms, including profuse sweating, labored breathing, hyperthermia, tachycardia, intense thirst, yellow staining of the skin, a sensation of feeling hot, and mental confusion. Muscular rigidity developed shortly before they died. Autopsies were performed 8 days later. The skin bore blisters filled with a dark red liquid, and the epidermis was edematous. Internal examinations of both corpses revealed dark-gray, friable, enlarged livers, dilated intestinal loops, darkly colored, friable lungs, and slate-colored spleens. The surviving brother was thirsty and perspiring when he entered

the clinic, and his skin was stained yellow. He returned home the same day, but his condition worsened. He lost the motor function of his legs, was dyspneic, exhibited signs of confusion, and sweated profusely. His body temperature was 39 C. He was readmitted to a hospital, and his condition improved gradually. He was released on the 8th day after the initial exposure had taken place.

Sovljanski et al [14], in 1971, described two cases of lethal intoxication from DNOC that occurred in Yugoslavia. Both involved farmers, aged 50-60 years, who were exposed while spraying fruit trees with DNOC. They used no personal protective equipment. One man's hands and clothes became yellow. After the day's work, he washed and went to sleep, but during the night he became comatose and was taken to the hospital the next morning. His pulse rate (126/minute) and blood pressure (170/95) were both elevated. The farmer's condition improved with treatment, and he was removed from intensive care after 7 days. There were still some signs of intoxication, however, including slow pupil response, increased tendon reflexes, and slightly excessive muscle tone. He was discharged from the hospital but died 3 days later. Death was attributed to choking on food eaten during breakfast.

In the second case recounted by Sovljanski et al [14], the circumstances were similar to those of the first, and death was also said to have resulted from choking on food. The authors thought that DNOC indirectly caused these deaths by impairing swallowing. This belief was not substantiated by any direct evidence, but microscopic examination of the brains after death showed that they had been affected. There were

signs of hemorrhage and infarction of the brain, areas of demyelination, and hyaline thrombi. The cells of the reticular formation showed chromatolysis. There was cytolysis of Purkinje cells and karyolysis of most brain cells.

Some authors have investigated the effectiveness of personal protective equipment in reducing exposure to DNOC. Burkatskaya [25], in conjunction with a study on the effects of airborne DNOC on cats (see Animal Toxicity section), examined the working conditions present in the manufacture and application of DNOC in Russia. He measured the concentrations of DNOC in the breathing zones of workers but did not describe the methods of sampling and analysis. Nonspecific effects on the workers were recorded. Workers exposed to DNOC at concentrations ranging from 0.0003 to 0.0029 mg/liter (average, 0.0009 mg/liter or 0.9 mg/cu m) displayed changes in the cardiovascular system, in the central and autonomic nervous systems, in the gastrointestinal tract, and in the cell pattern of the peripheral blood. The author did not describe the changes in detail. When DNOC was used agriculturally, its concentration in the air ranged from 0 to 0.013 mg/liter (average, 0.0007 mg/liter or 0.7 mg/cu m). Slight changes in the blood and autonomic nervous system were reported, but there were only isolated unspecified complaints from the workers. Since the author did not specify the changes that were observed, their magnitude and importance cannot be ascertained.

Van Noort et al [21] investigated the effectiveness of the personal protective equipment used by eight sprayers in May 1956. The authors measured the serum DNOC levels and recorded the quantity of DNOC used (expressed as the weight of DNOC) during the course of a 1-month spraying

period. From this study, they presented the results found in four workers. In one sprayer, who wore no protective devices, the serum DNOC level rose continuously during the spraying period, increasing at a rapid rate after the 1st week of exposure. By the end of 1 month, when he had sprayed over 800 kg of DNOC, his serum DNOC level was 64 μg/ml. A second worker wore gloves and a fresh-air hood during the spraying operation. Although he had sprayed 480.6 kg of DNOC in 23 days, his serum DNOC level reached no more than 16 μg/ml, indicating that the protective equipment offered protection but did not totally prevent contact with DNOC. Some of the workers who wore a fresh-air hood but no gloves absorbed little DNOC; others received an appreciable amount of the compound. In two of the sprayers from the latter group, the serum DNOC levels after 1 month were 65 μg/ml and 61 μg/ml. Each man had sprayed a total of 649 kg of the DNOC formulation. The authors did not report whether any signs or symptoms of intoxication had developed in the workers.

In May 1958, Van Noort et al [21] studied 24 sprayers to further examine the effectiveness of personal protective devices in preventing exposure to DNOC. Serum DNOC levels and the quantity of DNOC used were determined during a 3-week spraying period. The results from three workers were presented. In one worker who used no protective equipment, the serum DNOC level rose continuously during the spraying period, and by the end, after about 700 kg of DNOC had been sprayed, it was 75 μg/ml. Another sprayer, who wore both gloves and a plastic mask that he changed daily, had used a total of 650 kg of DNOC, but his serum DNOC level never rose above 10 μg/ml. A third worker, who used gloves carelessly and did not wear a mask, absorbed an appreciable amount of DNOC. By the end of the spraying

period, he had used about 450 kg of DNOC, and his DNOC serum level reached 65 μg/ml. In most of the sprayers there was an unexplained sudden rise in serum DNOC levels in the last days of the spraying period. The environmental temperature rose about 8 C in the last few days but the authors could not decide whether this was related to the increased serum DNOC levels.

Van Noort et al [21] also measured the serum DNOC levels in 10 of the 24 sprayers weekly for 2 months after the spraying period ended. They found that DNOC was eliminated from the serum slowly and that the rate varied from individual to individual. On the last day of the spraying period, serum DNOC levels ranged from 11 to 88 μg/ml, and 2-8 weeks elapsed before for DNOC was cleared from the serum. The amount of time needed for DNOC to be totally eliminated from the serum was directly related to the quantity of DNOC in the serum on the last day of the exposure period.

The findings by Van Noort et al [21] show that both inhalation of and dermal contact with DNOC can lead to appreciable absorption into the blood stream. A worker who wore a hood but no gloves and workers who wore gloves but no respiratory protection had serum DNOC levels of 61-65 μg/ml. In contrast, the use of equipment to protect against inhalation and dermal contact prevented appreciable accumulation of DNOC in the serum.

Batchelor and coworkers [27] attempted to ascertain the quantities of DNOC to which a group of spray operators, who used DNOC as a blossom-thinning agent, were exposed. The sprayed material was a slurry containing 19% of the sodium salt of DNOC, 5% sodium butyl naphthalenesulfonate, 2% sodium chromate, and small amounts of sodium chloride and sodium sulfate, which was then diluted with water to 0.02-0.08% DNOC. Spraying was done

during April and May when the temperature ranged from an average low of 37 F to an average high of 66 F. The workers were generally exposed for no more than 5 days, 6 hours/day. Dermal exposure was estimated from the amount of DNOC collected on absorbent pads placed on the forearms, shoulders, thighs, and the back and front of the necks of the spray operators. Some workers also wore respirators with collection filters so that respiratory exposures could be measured. Urine and plasma samples were taken from several workers to determine the concentrations of DNOC. Urinary DNOC levels were measured before, during, and after exposure, while plasma DNOC levels were checked within 24 hours and 7 and 11 days after the last exposure to DNOC. Plasma DNOC levels were measured in six workers exposed to DNOC for periods ranging from 5 to 48 hours.

The authors [27] found from examination of 300 pads that a worker was dermally exposed to an average of 63.2 mg of DNOC/hour, assuming that DNOC did not penetrate the clothing. The average respiratory exposure, calculated from 74 samples, was 0.4 mg of DNOC/hour. (Assuming that a worker inhales 28.6 liters of air/minute, which is a suggested minute volume for a 68.5 kg man doing light work [28], this corresponds to an airborne DNOC level of 0.23 mg/cu m.) Only small amounts of DNOC were detected in the urine. Of 183 samples tested, only 5 had DNOC levels greater than 0.5 ppm; these ranged from 0.6 to 1.3 ppm. Plasma DNOC levels were also low. One day after exposure ended, the plasma levels ranged from 1.4 to 4.0 ppm (approximately 1.4-4.0 μg/ml). After 7 days, the levels ranged from 1.6 to 4.3 ppm (1.6-4.3 μg/ml), and, by 11 days, they ranged from less than 1.0 to 2.7 ppm (1.0-2.7 μg/ml). The authors reported that workers showed no symptoms of intoxication.

Epidemiologic Studies

No reports of epidemiologic studies of persons exposed to DNOC were found in the literature.

Animal Toxicity

Animal studies have investigated the absorption of DNOC through the respiratory system [29,25,30], skin [15,26,31], and gastrointestinal tract [15,26,29,31,32] and the resulting blood levels and systemic effects. Authors have expressed blood DNOC values either as weight of DNOC/volume of serum or weight of DNOC/weight of whole blood. Parker et al [33] observed that over 90% of the DNOC detected in the blood was in the plasma and only small amounts were in the red blood cells. Because of this fact, numerically similar DNOC whole blood and serum values do not represent equivalent DNOC concentrations and should not be compared quantitatively. (In the following reports, the age, sex, and number of animals used in the experiments will be given if known).

(a) Inhalation

King and Harvey [29] exposed rats to a sublimate of DNOC, which acted as an aerosol. DNOC aerosol was generated by passing air at the rate of 1 liter/minute over an apparatus in which DNOC was heated, and 0.2 mg of sublimate was produced per hour. The aerosol generator and the cage containing the rats were enclosed in a glass chamber through which the airflow could be regulated and from which the outflow could be sampled for DNOC analysis.

In one experiment, four rats were exposed to DNOC aerosol at a

reported concentration of 0.1 mg/cu m for 5 hours [29]. The rats were removed at hourly intervals during the exposure, and blood samples were taken from the tail vein to measure DNOC levels. The rats were killed immediately after the exposure period, and DNOC was measured in the lungs and in the alimentary canal and its contents. The blood DNOC levels gradually rose during the exposure from about 20 μg/g after 1 hour to about 50 μg/g by the 5th hour. (These blood DNOC levels bring into question the reported airborne DNOC concentration. Even if one assumes that at a concentration of 0.1 mg/cu m all of the DNOC that was inhaled remained in the blood DNOC, a blood DNOC level of 20 μg/g could never be attained in 1 hour. In addition, the blood levels attained after exposure at 0.1 mg/cu m were similar to those attained at 100 mg/cu m, which is not likely, given the large difference in exposure concentrations.) The lungs of four rats contained 16, 20, 31, and 28 μg of DNOC/g, and the corresponding concentrations in the alimentary canal and contents were 2.5, 3.1, 2.8, and 2.2 μg of DNOC/g. These data suggested to the authors that the blood DNOC levels resulted mainly from inhalation of the aerosol and not from exposure by other routes. If there had been an appreciable amount of ingestion of contaminated food or water by the rats, DNOC levels in the alimentary canal would have probably been higher.

In a second inhalation experiment described by King and Harvey [29], five rats were exposed to DNOC aerosol at a concentration of 100 mg/cu m for 4 hours in a chamber maintained at 28-30 C. Respiratory rate, body temperature, and blood DNOC levels were measured hourly during the exposure period and again 20 hours after the exposure ended. The rats were kept at 20-22 C after they were removed from the chamber. In all five rats, blood

DNOC levels generally rose over the 4 hours, reaching a peak of between 16 and 64 μg/g in the 4th hour. Body temperatures decreased in the 1st hour in three rats and by the 4th hour it was lower than the preexposure level in three and was higher than the preexposure level in the other two animals. Respiratory rates varied during the 4 hours, but they were lower than the initial values in four of the five rats by the end of the period. In four rats, the blood DNOC levels were lower at 20 hours than they were immediately after exposure. Twenty hours after exposure, the blood concentrations of DNOC were between 17 and 29 μg/g in the five rats. The respiration rates had increased appreciably in three of the rats, and body temperatures had increased in two. The authors suggested that, although the compound was not accumulating in the blood after exposure ended, there might be an accumulation of the metabolic effects of DNOC, as indicated by delayed changes in respiration rates and body temperatures. However, the data were not conclusive since the results were so different in the five rats.

Burkatskaya [25] also examined the effects of DNOC inhalation by exposing 36 cats to either a liquid (fine dispersion of solution) or a solid (dispersion of solid) aerosol of DNOC in chambers that permitted exposure of the head only. The methods of generating the aerosols were not given in the report. Groups of three cats each received a single 4-hour exposure to the liquid aerosol at a concentration of 0.0004, 0.0014, or 0.04 mg/liter (0.4, 1.4, and 40 mg/cu m, respectively) or to the solid aerosol at a concentration of 0.036 or 0.06 mg/liter (36 and 60 mg/cu m). Six cats each received a single 4-hour exposure to the liquid or solid aerosol at a concentration of 0.1 mg/liter (100 mg/cu m). Three cats were

exposed for 4 hours daily for 1 month to the solid aerosol at 0.002 mg/liter (2 mg/cu m), and three cats each were exposed daily for 4 hours to the liquid aerosol at 0.0002 mg/liter (0.2 mg/cu m) for 2 or 3 months. Toxic effects were judged by changes in body weight, appetite, body temperature, cell counts, blood catalase and peroxidase activities, blood sugar levels, and erythrocyte sedimentation rate (ESR). The DNOC content of the blood or serum was also determined. In cats that showed toxic effects, measurements were made at unspecified intervals for up to 2 weeks after exposure ended. The author did not mention using controls in the experiment. Presumably, results obtained during the treatment periods were compared to preexposure values. It was not indicated whether measurements were made during the 1-month exposure or only after the exposure ended, but they were taken during the course of the 2- and 3-month experiments.

No deaths occurred in cats exposed to the liquid aerosol at a DNOC concentration of 1.4 mg/cu m or less or to the solid aerosol at 60 mg/cu m or less [25]. One cat exposed to the liquid aerosol at 40 mg/cu m and two cats exposed to the liquid aerosol at 100 mg/cu m died. The author noted that animals surviving the exposure at 40 mg/cu m exhibited increased body temperature, leukocyte count, and blood sugar concentration and decreased hemoglobin concentration, erythrocyte count, and catalase and peroxidase activities. Effects from a single 4-hour exposure to the liquid aerosol at 100 mg/cu m were variable. Catalase activity increased in three cats but decreased in the three others. Blood sugar concentration rose in four cats but remained unchanged in two. Peroxidase activity decreased in all six, while body temperature rose in only three. In cats exposed once to the solid aerosol of DNOC at 36 or 60 mg/cu m, some toxic signs were observed,

including salivation, lacrimation, spasms in the eyelids, sneezing, mucous nasal secretion, labored breathing, and sluggishness. Two cats also exhibited twitching, tremors, and ataxia. Hemoglobin concentrations and erythrocyte counts were decreased, while blood sugar was increased 20-25%. The ESR increased by 8-37 mm/hour. There were also slight decreases in peroxidase and catalase activities. Effects from exposure to the solid aerosol at 100 mg/cu m were similar in nature but more pronounced. Two of six cats exposed at this concentration died.

Blood and serum DNOC levels varied in the cats [25]. In cats that inhaled the liquid aerosol, blood DNOC levels were 2 mg% (20 μg/ml) in one on the 2nd day after exposure to 0.4 mg/cu m and 4 mg% (40 μg/ml) in the one that died after exposure at 40 mg/cu m. The DNOC serum level was 6-15 mg% (60-150 μg/ml) in those cats exposed to 100 mg/cu m. Blood DNOC levels of up to 10-15 mg% (100-150 μg/ml) were detected for 2 days after exposure to the solid aerosol at 36, 60, or 100 mg/cu m. DNOC was eliminated from the blood of all cats within 8 days of exposure.

A daily 4-hour exposure to DNOC at 2 mg/cu m for 1 month resulted in the deaths of two of three cats [25]. One died on the 26th day and one died 6 days after the exposure ended. Other changes noted were similar to those seen in cats exposed once to the solid aerosol. DNOC was not detected in the blood of these cats.

Cats exposed daily for 2 or 3 months to DNOC at a concentration of 0.2 mg/cu m had only slight increases in body temperature, leukocyte count, and ESR and slight decreases in hemoglobin concentration, erythrocyte count, and catalase and peroxidase activities [25]. Changes began to appear 1-2 weeks after exposure began, after which values remained stable.

None of the cats that were exposed at this concentration died. Blood DNOC levels of up to 1-2 mg% (10-20 μg/ml) were detected in two of the cats.

Burkatskaya [25] observed several effects from exposure to DNOC at concentrations as low as 0.2 mg/cu m, including changes in leukocyte and erythrocyte counts and peroxidase and catalase activities. The small number of cats in each exposure group and the absence of data for some of these groups makes the results questionable. However, the absence of these details does not invalidate the general conclusion that chronic exposure (2-3 months) of cats to DNOC at 0.2 mg/cu m produced slight changes in blood counts and enzyme activities.

Popov and colleagues [30] investigated the effects of DNOC administered simultaneously by the oral and the inhalation routes. Three groups of 20 albino rats each were given DNOC perorally (0.005 mg/kg), by inhalation (0.001 mg/cu m), or by combined exposure at 0.005 mg/kg perorally and 0.001 mg/cu m by inhalation. A fourth group of rats was used as controls. The control rats and those given DNOC perorally were kept in exposure chambers supplied with air. The exposure period lasted 2 months and included continuous exposure to DNOC or supplied air by inhalation and, for the appropriate groups, daily peroral administration. Observations and tests were made at 24 hours and 10, 30, and 60 days after the start of the exposure. They included monitoring of the rats' behavior and physical condition and measurements of oxygen consumption, erythrocyte and leukocyte counts, hemoglobin concentration, and the activities of the serum enzymes glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, lactic dehydrogenase, peroxidase, and catalase. The level of DNOC in the blood serum was measured in the middle and at the end of the

experiment. Although no specific data were presented, the authors reported that there were no adverse effects from DNOC at the concentrations administered.

(b) Dermal Exposure

The effects of DNOC applied to the skin of rats and rabbits were examined by Ambrose [15]. DNOC in a 2% aqueous solution (quantity not specified) was applied daily for 30 days to the depilated backs of 10 rats and to the depilated ventral surfaces of 6 rabbits. An unspecified number of rats served as controls. No signs of local irritation or systemic effects were evident in any of the animals given DNOC. The treated rats and the controls gained weight at similar rates.

Ambrose [15] also introduced five drops of a 1% aqueous solution of DNOC into the conjunctival sac of each of six other rabbits every 30 minutes for 6 hours. No signs of irritation were apparent during the 6-hour treatment period or 24 hours later.

The toxicity of DNOC in guinea pigs by dermal application was investigated by Spencer et al [34]. Single doses of 100-1,000 mg/kg of DNOC in an alcoholic solution were applied to the shaved abdomens of 27 guinea pigs. To facilitate absorption, the treated area of the skin was kept wet with ethanol for 4 hours after DNOC was applied. Deaths were recorded until the last surviving guinea pigs recovered fully from intoxication. This was the maximum observation period for recording deaths, provided full recovery could be defined satisfactorily. The authors found 200 mg/kg to be the highest "survival dose," ie, all five guinea pigs given this dose survived. The lowest "lethal dose" was 500 mg/kg; all five guinea pigs given this dose of DNOC died.

Burkatskaya [26] applied DNOC to the skin of rabbits in single doses ranging from 100 to 500 mg/kg in a dry form or as a "thick mush" and in single doses of 500 and 1,000 mg/kg as a 3% aqueous suspension. The procedure used for applying was not described. The concentration of DNOC in the blood was measured in rabbits given the aqueous suspensions of DNOC. DNOC applied in the dry form or as a thick mush produced no local irritation or systemic effects. The 500 mg/kg dose of the aqueous suspension also produced no local irritation, but it did cause changes in respiration, cardiac activity, and temperature regulation. The author did not describe the method used to measure those changes or specifically what was observed. DNOC was detected for 8-12 days at a concentration of 4-8 mg% (40-80 μg/ml) in the blood of the rabbits given 500 mg/kg of DNOC in an aqueous suspension. The 1,000 mg/kg dose of DNOC was reported to be the LD50. One hour after this dose was given, the blood levels were 2-4 mg% (20-40 μg/ml). The blood concentrations reached maximums of 10-40 mg% (100-400 μg/ml) and some of these rabbits died. The author reported finding no direct relationship between blood levels and the development of toxic effects.

Arustamyan [31] described the effects of an aqueous solution of DNOC applied to the skin on the backs of mice for an unspecified time. No mention was made of the use of control mice. The author observed the animals for signs of intoxication, recorded the number of deaths, and examined the internal organs following death.

The LD0 was determined to be 80 mg/kg, and the LD50 was 186.7 mg/kg [31]. Signs of poisoning included severe agitation, muscular twitching, labored breathing, extreme thirst, and loss of appetite. Post-mortem

examination of the mice revealed enlarged livers with spot hemorrhages and necrotic foci, spleens twice the normal size, and pulmonary edema. Cutaneous application of the aqueous suspension of DNOC did not produce skin irritation.

(c) Other Routes of Administration

The acute toxicity of DNOC given by subcutaneous injection has been investigated in a number of studies [15,17,35]. Ambrose [15] reported that the minimum fatal dose of DNOC in male and female rats (100-125 g) was 20 mg/kg. Signs of intoxication included hyperactivity followed by depression, labored breathing, asphyxial convulsions, and coma. Rigor mortis developed immediately after death. Parker et al [33] found that the LD50 of 1% DNOC in a weak sodium carbonate solution was 24.6 mg/kg in rats and 24.2 mg/kg in mice. Intoxicated animals exhibited increased respiratory rate and prostration. Rigor mortis was apparent immediately after death. Harvey [35] found that the LD50 values in albino rats for pure DNOC and for four commercial samples of DNOC were 25.6 and 26.2-27.5 mg/kg, respectively. The samples were prepared as 1% (w/v) saline solutions. In hooded rats, the LD50 values were 28.5, 27.5-30.0, and 36.5-39.1 mg/kg for pure DNOC, a commercial preparation, and the diethylamine salt of DNOC, respectively [35].

Some investigators have studied the toxicity of DNOC administered by stomach tube [15,26,31,32,34]. According to Ambrose [15], the minimum fatal dose of DNOC for 150 g male rats was 30 mg/kg. Signs of intoxication were similar to those observed in rats given DNOC by subcutaneous injection. Burkatskaya [26] reported that the LD50 values in mice, rats, and cats were 47, 85, and 50 mg/kg, respectively. Signs of intoxication

observed in the cats included accelerated breathing, salivation, sluggishness, muscular twitching, and loss of appetite. Arustamyan [31] determined that the single dose oral LD50 in mice was 16.4 mg/kg. Signs of intoxication included severe agitation, muscular twitching, thirst, and refusal of food. Post-mortem examination revealed enlarged livers and spleens and inflammation of the intestinal mucosa. The single dose oral LD50 values for DNOC, given as a 35% solution of its ammonium salt, were 8.4, 8.3, and 24.8 mg/kg for pheasants, partridges, and hares, respectively [32]. Poisoned animals exhibited heavy breathing, asphyxial spasms, and muscular rigidity. Edema and emphysema of the lungs, dilation of the heart, inflammation of the mucous membranes of the intestines, and changes in kidney cell morphology were found in animals that died. Spencer et al [34] examined the effects of DNOC given in olive oil to 100 white rats of both sexes by stomach tube. The largest single dose survived by all rats so treated was 10 mg/kg, while 50 mg/kg was the smallest dose that produced 100% mortality. The authors attributed death to the pyretic effect of DNOC.

The distribution of DNOC in the body tissues and its cumulative properties have been studied using the subcutaneous, intraperitoneal, and oral routes of administration [15,29,32,33]. Ambrose [15] gave 20 rats, weighing about 100 g each, daily subcutaneous injections of 15 mg/kg of DNOC for 30 days. Controls were used, but the author did not describe the experimental conditions for this group. The urine was examined at unspecified intervals for glucose, albumin, and red cells. Hemoglobin concentrations and red blood cell counts were determined at the start of the experiment and on the day after the last injection. After 30 days, the

rats were killed, and portions of the urinary bladder, stomach, small intestine, kidneys, liver, spleen, heart, and lungs were examined microscopically. No toxic signs developed in the animals given DNOC, and the urinary and blood findings were no different from those of the controls. Rats given DNOC had a slightly larger mean increase in body weight (46%) than the controls (42%) during the 30-day period. No changes were observed in those tissues examined microscopically. The author concluded that daily sublethal doses of DNOC did not accumulate in rats.

Parker et al [33] observed the effects on rats and rabbits of 5 mg/kg doses of DNOC injected subcutaneously at 1-hour intervals. The DNOC was given as a 1% solution in weak sodium carbonate solution. A single 5 mg/kg dose produced no signs of poisoning in either rats or rabbits; however, some animals died after the fifth to seventh injections. Each animal received a total of 25-35 mg/kg, an amount similar to the LD50 for a single subcutaneous injection. This indicated to the authors that DNOC accumulated in the animals, but the total dose was given over only 5-7 hours. The authors also gave dogs subcutaneous injections of 5 or 10 mg/kg of DNOC daily for 12 consecutive days. They stated that there was no evidence of an accumulation of toxic effects from repeated doses of DNOC.

King and Harvey [29] investigated the cumulative properties of DNOC given in repeated daily doses to rats weighing 104-253 g and to rabbits weighing 1,400-1,805 g. DNOC was given by stomach tube for 8 consecutive days to six female rabbits at 25 mg/kg/day, to six female rats at 20 mg/kg/day, to six male rats at 10 mg/kg/day, and to five male rats at 5 mg/kg/day. DNOC was also administered by daily intraperitoneal (ip) injection for 14 days to three male and three female rats at 10 mg/kg/day

and to three male and three female rats at 5 mg/kg/day. The blood concentration of DNOC was measured 24 hours after each dose was given.

After the second daily dose of DNOC was given to the rats by either route, the blood concentration of DNOC was significantly higher than it was after the first dose [29]. Succeeding ip doses did not produce blood levels higher than those observed after the second dose. However, succeeding doses of 5 and 10 mg/kg by stomach tube caused small increases in the blood levels from the 3rd day onward, while the 20 mg/kg dose produced results similar to those caused by the ip injections. The authors did not speculate about these differences in the rats being related to the sex differences in the dose groups. In the rabbits, the DNOC level in the blood was no greater after the second dose than after the first.

Janda [32] gave pheasants and partridges three daily doses of DNOC by stomach tube as a 35% solution of the ammonium salt. The LD50 values, expressed in terms of the daily dose, were 7.1 mg/kg/day and 11.1 mg/kg/day for the pheasant and partridge, respectively. These LD50's were similar to those reported by Janda for a single dose oral LD50 of DNOC (8.4 and 8.3 mg/kg) and suggest that DNOC is eliminated rapidly from the pheasant and partridge.

Parker et al [33] found that DNOC injected subcutaneously disappeared from the blood at various rates in different species. Single 10 mg/kg doses of DNOC were administered subcutaneously to an unspecified number of dogs, cats, rabbits, and rats. Serum concentrations of DNOC were measured daily for 6 days after the injection. Other rats, rabbits, and dogs were given daily 10 mg/kg doses of DNOC, and the concentration of DNOC in the serum was measured 24 hours after each injection. The rats were given two

injections, and the rabbits and dogs were each given five. DNOC given in one injection was completely eliminated from the serum of rabbits within 24 hours, while blood DNOC levels were between 30 and 40 μg/ml in the rats, cats, and dogs at this time. It took 4 days for DNOC levels to fall to zero in rats and cats, and 6 days in dogs. The authors reported that serum levels of DNOC were no higher on succeeding days in rats, rabbits, and dogs that received daily doses than they were 24 hours after the first dose. However, that statement contradicted the data presented for the dogs, which showed that the serum DNOC level rose from about 45 μg/ml 24 hours after the first dose to 60 μg/ml after the second dose and peaked at 67 μg/ml after the third dose. The DNOC concentration then began to fall, although two additional doses were given to the dogs. Six days after the fifth dose, the concentration was about 10 μg/ml.

The distribution of DNOC was measured in various tissues in animals given either single or repeated injections of DNOC. In one experiment, Parker et al [33] gave rats 1.5 mg of DNOC by subcutaneous injection, and one or more were killed 0.5, 1, 2, 3, 4, 5, and 6 hours after the dose was administered. Most of the DNOC that was recovered in the rats appeared in the serum. In the one rat killed 30 minutes after being given 1.5 mg of DNOC, 0.725 mg was recovered; 83% of this was found in the serum. From rats killed 6 hours after the injection, an average of 0.37 mg of DNOC was recovered; 72% of this was in the serum. Small amounts of DNOC were also found in the heart, lungs, kidneys, liver, and spleen.

In another experiment, the authors [33] gave single subcutaneous injections of 20 mg/kg of DNOC to 19 rats and 40 daily injections of the same dose to 9 rats. They found that the mean DNOC concentration in the

serum of the rats 24 hours after the single injection was 45±1.6 μg/g, which was similar to that measured 24 hours after the last of 40 injections. A similar pattern was observed in the liver and kidneys, although the mean DNOC concentrations in these organs were much smaller than that found in the serum.

These authors [33] also measured the excretion of DNOC in the urine of dogs and rabbits given single or repeated subcutaneous injections of DNOC. The total quantity that was administered ranged from 0.5 to 80 mg. Urine was collected during the time injections were being given and for 3 days after the last injection. Between 4 and 10% of the total amount of DNOC injected was recovered in the urine as DNOC, and there was no difference in the percentage recovered from rabbits and from dogs.

King and Harvey [29] measured blood levels of DNOC in rats and rabbits that were exposed to DNOC by various routes of administration. They gave each of 12 albino rats a single 30 mg/kg dose of DNOC in 0.9% saline by stomach tube and then measured the distribution of the compound in the rats' bodies. Two rats were killed at each of the following intervals: 1, 2, 4, 7, 24, and 48 hours after the dose. The blood, stomach, small intestine, large intestine, and contents of the alimentary canal were analyzed for DNOC. An average of 19% of the dose was recovered in these tissues 1 hour after the exposure; 12% was recovered after 2 hours, and 3% after 7 hours. By 48 hours, only the large intestine and the alimentary canal contents contained appreciable amounts of DNOC. Blood DNOC levels reached a peak of about 50 μg/g between 2 and 7 hours after the single dose was given.

An additional 17 rats were given DNOC by stomach tube in single doses of 5, 10, 20, 40, 50, or 100 mg/kg [29]. Twelve rats were given the 40 mg/kg dose, and one rat each received the other doses. Samples of blood were taken from tail veins, and DNOC was measured at various intervals for up to 24 hours after the doses were given. One rabbit was given a single 10 mg/kg dose by stomach tube and a second was given 20 mg/kg. Blood samples were taken from their ears 2, 4, 6, 8, 12, and 24 hours later, and blood DNOC levels were measured. In both the rats and the rabbits, DNOC concentrations in the blood peaked within 8 hours; however, by 24 hours the levels were much lower in the rabbits than in the rats. Twenty-four hours after the 10 mg/kg dose was given, the blood levels were 25 μg/g in the rat and 4 μg/g in the rabbit. The blood level was 2.5 μg/g in the rabbit given 20 mg/kg. The rat that received this dose died before the 24-hour measurement could be made, but, even in the rat given 5 mg/kg, the blood DNOC level was higher than in the rabbits given 10 or 20 mg/kg. It was 12 μg/g after 24 hours. Maximum blood levels of 101, 92, and 88 μg/g were attained after administration of the 40, 50 and 100 mg/kg doses, respectively.

Four rats were each given one ip injection of DNOC at doses of 1, 5, 10, or 20 mg/kg [29]. Blood levels of DNOC were measured 1, 3, 5, 8, 11, and 27 hours after the doses were given. The injections generally produced higher blood concentrations of DNOC that peaked at a faster rate than did similar doses given to rats by stomach tube. This was especially evident at a dose of 20 mg/kg.

Several studies have been conducted to investigate the effect of environmental temperature on the toxicity of DNOC [29,33,36]. The

experimenters have given DNOC by various routes of exposure to several different animal species.

The effects of environmental temperature on the toxicity of DNOC and on the accumulation of DNOC in the blood were studied in rats by King and Harvey [29]. Groups of 24 rats each were given DNOC in single doses ranging from 5 to 50 mg/kg by stomach tube; 12 rats at each dose were kept at 20-22 C and 12 at 37-40 C. After receiving a 20 mg/kg dose, all of the rats at 20 C, but only half of those at 37 C, survived. Of the rats given 40 mg/kg, 16% of those kept at 20 C and all of those kept at 37 C died. At 50 mg/kg, 33 and 100% of the rats kept at 20 C and 37 C, respectively, died. An analysis of the data indicates that the approximate LD50 values for rats kept at 20-22 C and 37-40 C were 55 and 20 mg/kg, respectively. The authors also reported that blood DNOC levels did not differ in groups of hooded rats kept at different environmental temperatures following a single oral dose of 40 mg/kg.

King and Harvey [29] obtained inconsistent results in their studies of the effects of environmental temperature on blood DNOC levels in rabbits given DNOC dermally. In one experiment, blood DNOC levels were no different in two groups of rabbits kept at different temperatures. However, in another experiment, the authors found that blood DNOC levels rose after a group of rabbits was moved to a warm room.

Other investigators have observed increases in the toxicity of DNOC with increasing environmental temperatures. The LD50 for a single subcutaneous injection of DNOC was lower in rats kept at 36-37 C than in rats at 5-10 C [33]. Mice given a subcutaneous injection of DNOC and kept at 40 C died in a shorter time than those kept at 10 or 20 C [36].

Vashakidze [37] investigated the effect of DNOC administration on the reproductive cycle of rats. In one experiment, female rats (weight and number unspecified) were given DNOC by mouth repeatedly for 6 months (exact regimen not reported) in doses of 2, 5, or 10 mg/kg. At the end of 6 months, hypophyseal suspensions of unspecified concentration were prepared from these rats and administered by an unstated route to immature female rats weighing 15-20 g. One control group of immature female rats received hypophyseal suspensions from untreated donor rats, and a second control group of immature female rats received no suspension. The gonadotropic activity of the hypophyses of DNOC-treated rats was measured by monitoring the development of the sexual organs in the recipient rats. Toxic effects on the donor rats were also monitored.

Donor rats that received 10 mg/kg of DNOC exhibited a 10-18% lag in weight gain, fatty degeneration of some organs, various disorders in the functioning of the reproductive glands, and a reduction in the number of phases of heat [37]. The 2 and 5 mg/kg doses did not produce this spectrum of toxic effects, although the 5 mg/kg dose had an unspecified effect on the reproductive glands. When immature rats were given hypophyseal suspensions from rats treated with repeated 10 mg/kg doses of DNOC, the average weights of the uteri and ovaries of the recipient rats were 38 and 36% greater, respectively, than those of the rats that were given hypophyseal suspensions from untreated donors. This demonstrated an increased activity of gonadotropin in the pituitaries of rats treated with 10 mg/kg of DNOC. Hypophyseal suspensions from rats given the 2 and 5 mg/kg doses produced slight or no change in the weights of the ovaries or

uteri of the immature rats. Results from the second control group were not reported.

In a second experiment, Vashakidze [37] studied the reactivity of the vaginal mucosa in rats following poisoning with DNOC. The protocol of this experiment is unclear. Apparently, immature female rats were treated with DNOC in doses of 2, 5, or 10 mg/kg for 6 months. At the end of this time, they were then treated for 5 days with 5,000 units of folliculin (estrogen). A second group of immature rats received no DNOC but were given the same amount of folliculin 1 month after having their ovaries removed. Control rats, either given DNOC or ovariectomized, received injections of olive oil instead of folliculin. Vashakidze reported that a dose of 10 mg/kg of DNOC disrupted the reactivity of the vaginal mucosa to folliculin in about 10% of the rats, as determined by vaginal smears. At lower doses, no disruption was seen. Results for the ovariectomized animals were not reported.

In a third experiment, Vashakidze [37] gave an unstated number of female rats DNOC repeatedly for 6 months in doses of 2, 5, or 10 mg/kg, which produced disruption of the estrous cycle. These rats were then given transplanted ovaries from untreated 3-week-old rats. Transplants were also given to rats that had been surgically ovariectomized. Transplanting of ovaries to rats that had been given DNOC resulted in restoration of the estrous cycle in 70-90% of the rats within 20 days. Transplantation of ovaries also restored the appearance of the body and horns of the uterus, which had atrophied following DNOC poisoning. When the transplanted ovaries were removed, cycling stopped in most animals (number unspecified). Where cycling continued, the author attributed this to a restoration in the

functioning of the animals' own ovaries and to a residual influence of the transplants. In ovariectomized animals, transplantation of ovaries restored cycling within 20-40 days. The number of rats that continued to cycle, however, decreased in the next few months because the ovaries were resorbed. Vashakidze [37] concluded, particularly from the data of the third experiment, that DNOC disrupted the reproductive cycle by direct action on the ovaries. However, because of the experimental design and because the data were not clearly presented, it is difficult to substantiate this conclusion.

Kreczko et al [38] studied the effect of DNOC on glycoprotein biosynthesis in guinea pigs. Thirty male guinea pigs, 8-12 months old and weighing 350-400 g, were each given a 9 mg/kg dose of DNOC in 0.5 ml of 0.9% NaCl solution ip six times/week over a period of 30 days. Twenty control guinea pigs were given injections of 0.5 ml of saline according to the same regimen. The guinea pigs were killed 24 hours after the last injection. Total sialic acid and amino sugar concentrations in the liver and in the serum were measured. Glycoprotein fractions of the serum were separated electrophoretically and measured, as was the DNOC concentration in the blood.

The authors [38] found that blood levels of DNOC in guinea pigs given injections of DNOC ranged from 21 to 32 μg/ml. No DNOC was found in the blood of the control guinea pigs. Amino sugar and sialic acid concentrations were significantly greater in the liver and serum of guinea pigs given DNOC than in the controls, and the glycoprotein concentrations of the albumin and alpha-2 globulin fractions of their serum were lower than in controls. The investigators also found increases in the

glycoprotein content of the serum alpha-1 and gamma globulin fractions and a slight increase in the glycoprotein content of the beta globulin fraction of the guinea pigs given DNOC. These findings suggested to the authors that DNOC might selectively block the synthesis of some glycoproteins while simultaneously increasing the production of those that contain high amounts of sugar, such as alpha-1 globulin.

Burkatskaya and Karpenko [39] investigated the effect of DNOC on white rats, especially on the levels of sodium and potassium in various tissues. Eighty rats weighing 150-200 g were divided into five groups and an unspecified number in one group were each given one oral dose of 50 mg of DNOC/kg of body weight. A group of 38 rats served as controls. The sex of the rats was not stated. Signs of intoxication were recorded for 2 hours after the dose was administered, and the animals were then killed. The authors measured the levels of sodium and potassium in the blood plasma, erythrocytes, myocardium, liver, and kidneys, the concentration of blood sugar, and the distribution of water in the myocardium and liver.

Toxic effects were observed in 30% of the rats that were given DNOC, but specific signs were not mentioned [39]. Both electrolyte levels were significantly higher ($P<0.05$) in the plasma and kidneys of the rats given DNOC than in the tissues from control animals. DNOC administration produced a significant decrease ($P<0.05$) in the potassium level in the myocardium and a significant increase ($P<0.05$) in the sodium content of the myocardium and liver. The concentrations of both elements in the erythrocytes and of potassium in the liver were unchanged. The rats were hyperglycemic after being given DNOC, but the actual blood sugar level was not specified. The authors found significant changes ($P<0.05$) in the

distribution of water in the cells. There was an increase in the intracellular water, a decrease in the extracellular water, and a decrease in the total water in the myocardium and liver following DNOC administration.

Many cases of cataract formation in people taking DNOC internally for weight reduction have been reported [7,12]. Because of this observation, Spencer et al [34] investigated the ability of DNOC to produce cataracts in animals. Ducklings had previously been found to be susceptible to developing cataracts following dinitrophenol exposure, and were therefore used in the experiment. A diet containing 0.25% DNOC was given to 8-10 2-week-old ducklings. Within 24 hours, all of the birds had developed cataracts, and by the next day they were all dead.

Only one report was found in the literature in which the mutagenic potential of DNOC was investigated. In 1972, Andersen et al [40] reported an evaluation of the ability of 110 herbicides, including DNOC, to produce point mutations in histidine-dependent mutants of Salmonella typhimurium, bacteriophage T4, and in two RII mutants of bacteriophage T4. The culture mediums were prepared by mixing freshly grown cultures of the mutants with soft agar and pouring into petri dishes. After the agar solidifed, DNOC was applied to the surface of each plate. They found that the mutation frequency rates produced by DNOC were no greater than the spontaneous rates. Although the data from this report indicate that DNOC is not mutagenic in a spot test, the experiment was not adequate for fully evaluating the mutagenicity of DNOC. The actual Salmonella strains were not identified and liver postmitochondrial activation systems were not utilized, thereby preventing consideration of possible activation of DNOC.

The success of the test relied on DNOC diffusing into the medium, since DNOC was not incorporated with the agar.

In several of the human studies described in this chapter, the investigators [6,8,10-13,17,19,21-23] have reported that one major effect observed in individuals exposed to DNOC is a large increase in the BMR. Some investigators have concluded that DNOC affects metabolism by uncoupling the oxidative phosphorylation process [21,22,41], resulting in increased cellular respiration (increased oxygen consumption) and decreased formation of adenosine triphosphate (ATP), which contains "high-energy" phosphate bonds. Therefore, energy generated in the body cannot be converted to its usual form (ATP) and is released as heat instead [33].

The effects of DNOC on the oxidative phosphorylation process have been investigated by both in vivo and in vitro techniques [42,43]. Muscatello et al [42] measured the rate of oxygen uptake and the activity of adenosine triphosphatase (ATPase) in liver mitochrondria taken from fasted male rats weighing 200-300 g. They found that DNOC at a concentration of 5 μM in a mitochondrial preparation produced a maximum increase in oxygen uptake and also increased ATPase activity.

Burkatskaya and Anina [43] gave rats, weighing 150-170 g, 75 mg/kg of DNOC orally and observed the effects on oxidative phosphorylation. As soon as the rats exhibited toxic effects, such as increased respiratory rate, they were killed. Another group of rats served as controls, but the control conditions were not specified. Livers were removed, and the concentration of ATP in liver tissue was determined. The ATP content was 188±21 μg of phosphorus/g of tissue in controls and 34±5 μg of phosphorus/g of tissue in rats exposed to DNOC.

Lehninger [44], in Biochemistry, states that uncoupling agents stimulate oxygen uptake and cause increases in the ATP-hydrolyzing activity. Increases in ATPase, as observed by Muscatello et al [42], would indicate that an increased rate of breakdown of ATP to adenosine diphosphate (ADP) is occurring.

Muscatello et al [42] also examined the ultrastructure of mitochondria by electron microscopy after DNOC was added to mitochondrial suspensions prepared from rat livers. They observed that DNOC caused the mitochondria to condense and form invaginations of the inner membrane and produced a significant decrease in the total volume of the mitochondria. However, since the authors found that other factors influenced mitochondrial configurations, they could not directly relate such changes to an uncoupling effect. The finding of configurational changes might be related to the coupling of oxidative phosphorylation that, according to one theory, occurs through conformational changes in the structure of the mitochondria [44].

(d) Metabolism

Several investigators have conducted experiments to determine the fate of DNOC after its administration to animals [45-47]. Urinary metabolites have been measured, and a detoxification pathway for DNOC has been suggested.

Truhaut and De Lavaur [45] reported on the metabolism of DNOC in rabbits. The compound was administered as an alkaline aqueous solution by gastric intubation to rabbits. Doses of 5, 10, 15, 18, and 20 mg/kg were administered to the rabbits, which had been fasted for 15 hours. The distribution of DNOC and its aminonitro metabolites was examined in the

blood, bone marrow, adipose tissue, kidneys, liver, brain, and urine within 7 hours after the dose was given.

When 10 and 15 mg of DNOC/kg were given to rabbits, the blood DNOC concentrations rose to 25 and 34 μg/g, respectively, and remained at those levels for 5-6 hours [45]. A dose of 18 mg/kg, which was fatal to the rabbit, produced a blood DNOC level of 50 μg/g. No amino derivatives were detected in the blood, the bone marrow, or the adipose tissue of the animals. However, 6-amino-4-nitro-o-cresol was detected in the liver, kidneys, and brain, while no 4-amino-6-nitro-o-cresol was found. It was concluded that the ratio of 6-amino-4-nitro-o-cresol to DNOC was a function of the dose of DNOC administered to the animal. When a low dose of DNOC (10 mg/kg) was given, little 6-amino-4-nitro-o-cresol was found in the kidneys, and none was in the liver and brain. As the dose of DNOC was increased, the ratio also increased, and it was especially high in the kidneys. An increase in the dose from 10 to 20 mg/kg raised the ratio in the kidneys from 0.42 to 5.29. Both DNOC and 6-amino-4-nitro-o-cresol were detected in the urine. When 10-15 mg/g were given, 25-38% of both metabolites were recovered in the urine. From 82 to 97% of this was eliminated in 24 hours, and the remainder in the next 2-3 days. As in the kidney, the ratio of 6-amino-4-nitro-o-cresol to DNOC in the urine increased as the dose of DNOC was increased, at least within the first 7 hours after the dose was administered. The ratios, measured 2.5-3.75 hours after doses of 10 and 20 mg/kg were given, were 0.66 and 1.47, respectively. The highest ratio measured was in a rabbit that died 3 hours after receiving DNOC. This animal was given an 18 mg/kg dose of DNOC, and the urinary ratio measured at the time of death was 2.40. Only small

amounts of 4-amino-6-nitro-o-cresol were detected in the urine. The authors considered the metabolism of DNOC to 6-amino-4-nitro-o-cresol a detoxification mechanism that plays an important role only when a toxic dose of DNOC is administered. They suggested that the ratio of 6-amino-4-nitro-o-cresol to DNOC might be a useful indicator in evaluating the severity of the exposure to DNOC.

The metabolic fate of DNOC in rabbits was also investigated by Smith et al [46]. Forty rabbits (sex and age not specified) were given 20-30 mg/kg of DNOC by stomach tube, and urine was collected from each for 2 days to measure metabolites. Three separate extracts of the urine samples were prepared, and metabolites were identified by paper chromatography and spectrophotometry.

Less than 20% of the dose of DNOC was recovered in the urine in 2 days [46]. Between 5 and 5.5% was excreted as free DNOC, and 0.7% as DNOC conjugates. The authors did not identify the conjugates more specifically. Most of the urinary metabolites (about 12% of the dose) were derivatives of 6-amino-4-nitro-o-cresol. About 1.5% of the dose was excreted as 6-acetamido-4-nitro-o-cresol and 9-10.6% as the hydroxyl group conjugate. Traces of 6-amino-4-nitro-o-cresol, 4-amino-6-nitro-o-cresol, and 3-amino-5-nitro-salicylic acid were also detected. The metabolic pathways of DNOC in the rabbit suggested by Smith et al are summarized in Figure XIII-1.

The authors [46] speculated that conversion of DNOC to 6-acetamido-4-nitro-o-cresol was the major detoxification pathway. They found that rabbits given 600 mg/kg of 6-amino-4-nitro-o-cresol or 2 g of 6-acetamido-4-nitro-o-cresol by stomach tube survived, while two rabbits died after being given 31 and 32 mg/kg of DNOC.

In 1970, Jegatheeswaran and Harvey [47] reported that the rumen microflora of sheep affect the metabolism of DNOC. In a preliminary experiment, they found that isolated rumen contents were able to reduce DNOC to 6-amino-4-nitro-o-cresol, which was then converted to 4,6-diamino-o-cresol. Separation of the rumen contents into three fractions (protozoa, bacteria, and cell-free supernatant) showed that only the first two fractions were able to reduce DNOC.

In a second experiment, 20 mg/kg of DNOC was given to one sheep by mouth and to another by ip injection [47]. Blood and urine samples were taken at various intervals for 3 days to measure the DNOC content. Administration by the ip route resulted in a tenfold greater serum level of DNOC than was produced by oral administration within hours of administration. The serum levels reached a peak in 4-6 hours, 110 μg/ml of serum for the ip route and 12 μg/ml of serum for the oral route. By 72 hours, the serum level was less than 10 μg/ml in both sheep. The type of metabolites found in the urine depended on the route of administration. Of the DNOC given by ip injection, 33.6% was accounted for in the urine, 3.7% as free DNOC, 7.2% as conjugated DNOC, 22.7% as conjugated 6-amino-4-nitro-o-cresol, and traces of 4,6-diamino-o-cresol. Of an oral dose, 30.6% was accounted for in the urine, 5.3% as conjugated 6-amino-4-nitro-o-cresol and 25.3% as 4,6-diamino-o-cresol.

Correlation of Exposure and Effect

The available literature concerning humans indicates that DNOC is absorbed through the respiratory and gastrointestinal tracts and through

the skin and that it accumulates in the blood. The signs and symptoms of intoxication observed in individuals exposed to DNOC by these routes were often associated with increased metabolism [6,9-13,17,19,21-23]. Effects included profuse sweating, thirst, lassitude, malaise, headache, loss of weight, a sensation of heat, and an increased metabolic rate. In addition, peoples' skin, hair, sclera, and conjunctiva have been stained yellow [6,9-14,16,17,19,21,22,24]. Apparently, although this staining indicated that exposure to DNOC had occurred, it provided no measure of the extent of biologic impairment [17,22].

In evaluating studies on manufacturing and agricultural workers, it was often difficult to assess the impact of inhalation versus dermal exposure unless a worker was using protective equipment that prevented exposure by a particular route. In addition to the toxic effects associated with increased metabolism, other reported effects in workers included kidney damage [20], diarrhea [22], unspecified changes in the gastrointestinal tract [25] and in the cardiovascular system and peripheral blood [25], and CNS disturbances, such as confusion [24], loss of motor function in the legs [24], visual disturbance [20], tingling sensations in the limbs [23], unspecified changes in the central and autonomic nervous systems [25], and microscopic changes in the brain [14]. Because the DNOC air concentrations in these studies were only rarely reported, no dose-response relationship could be determined.

Bidstrup and Payne [6] observed the profound effect of environmental temperature on DNOC toxicity. They noted that the only deaths in workers from DNOC exposure in Great Britain occurred in what the authors considered "unusually hot" weather (56-86 F). Other investigators have also reported

that the toxicity of DNOC in humans is enhanced during warm weather [17,21,22]. Investigations in mice [36] and rats [33] given DNOC subcutaneously, in rats given DNOC by stomach tube [29], and in rabbits with DNOC applied to the skin [29] have verified the influence of temperature on DNOC toxicity. Increasing the environmental temperature resulted in a decrease in the LD50 [29,33] and a decrease in the LT50 [36]. The effects of environmental temperature on DNOC toxicity in animals are summarized in Table III-1.

Studies in various animal species also have confirmed the toxicity of DNOC in humans exposed by the inhalation and dermal routes. King and Harvey [29] reported that rats exposed to DNOC aerosol at a concentration of 100 mg/cu m for 4 hours had a decreased respiration rate during the exposure period and that body temperature decreased in some but increased in others. In cats exposed to DNOC aerosol at concentrations of 36-100 mg/cu m for 4 hours, effects included salivation, lacrimation, labored breathing, sluggishness, tremors, increases in body temperature, blood sugar concentration, and leukocyte count, and decreases in hemoglobin concentration, erythrocyte count, and catalase and peroxidase activities [25]. At similar concentrations, repeated exposure to DNOC was more toxic than a single exposure [25]. Changes in blood cell counts and in catalase and peroxidase activities, which were categorized by the author as slight and transient, were found in cats exposed for 2 or 3 months at a DNOC air concentration of 0.2 mg/cu m. In contrast, no effects were observed after a single 4-hour exposure at 0.4 mg/cu m.

Conflicting evidence has been reported concerning the effects of dermal absorption of DNOC when this was the only route of exposure. One

author observed no effects in volunteers who had a 2% aqueous solution of DNOC applied to their shaved armpits and forearms daily for 30 days [15]. However, other studies [16,21,23] have shown that DNOC is absorbed through the skin and can produce both local and systemic effects. DNOC has been found in the blood after dermal application [13,21], and toxic effects have been observed, including peripheral neuritis [23] and death [16].

Similarly, reports of effects on animals from dermal exposure to DNOC have been conflicting. No effects were noticed in rats or rabbits given DNOC as a 2% aqueous solution for 30 days [15] or in rabbits given a single dose of DNOC in dry form [26]. A single application of DNOC as a 3% aqueous suspension produced unspecified changes in respiration, cardiac activity, and temperature regulation in rabbits [26]. Single dermal applications of DNOC as aqueous solutions produced agitation, twitching, labored breathing, thirst, and loss of appetite in mice [31], and applications as alcohol solutions produced death in guinea pigs [34]. The above mentioned human and animal experimental data [16,21,23,26,31,34] taken together indicate that dermal exposure to DNOC can lead to adverse health effects.

In studies conducted to determine the kinetics of absorption and distribution patterns, DNOC has not been shown to accumulate in the blood of various animal species [29,33]. In rats and rabbits that were given two or more daily subcutaneous injections of DNOC, the serum levels on succeeding days were no higher than they were 24 hours after the first dose [33]. In dogs, the serum level rose for the first 3 days but then fell, even though two additional doses were given [33]. DNOC was also eliminated from the blood of animals faster than it was in humans [29,33]. Within 24

hours after a single subcutaneous injection of DNOC, it was almost completely eliminated from the serum of rabbits. It took 4 days to be cleared from the serum of rats and cats and 6 days from dogs [33]. DNOC accumulated only slightly in the blood when given to rats by stomach tube or ip injection and did not accumulate in the blood when given to rabbits by stomach tube [29].

Inhalation studies in rats [29] have confirmed that DNOC is absorbed through the respiratory tract into the bloodstream but does not accumulate in the blood. The inhalation data in one report [25] also corroborated the findings in animals exposed to DNOC by other routes [29,33] that DNOC is eliminated from animals more quickly than from humans.

Although few of the reports found indicated the concentration of airborne DNOC at which signs and symptoms of intoxication occurred in humans, several studies have associated blood DNOC levels with toxic effects [13,17,19-21] and have shown that, unlike in animals, DNOC accumulates in the blood of humans. In comparing studies on blood DNOC levels, certain precautions must be taken when correlating the results. It was reported that over 90% of the DNOC detected in the blood was found in the serum [33]. Therefore, a comparison of numerically similar blood DNOC levels expressed as weight/volume of serum with those expressed as weight/weight of whole blood can only be done by approximate conversions. A given DNOC serum level will have a lower value when expressed as the amount in whole blood.

Another factor considered in comparing results was the time after exposure when blood DNOC levels were determined. Because DNOC is eliminated slowly from the human body [13,17,19,21], blood DNOC levels did

not vary much in the first few days. However, if the time interval from exposure to sample collection is longer than a few days, a comparison of results might not be valid.

Only one report [26] found in the literature documented both the blood DNOC levels in workers and the concentration of DNOC in the air they breathed. Insufficient data are available to determine a correlation between these two factors. The average DNOC concentration in the blood of agricultural sprayers ranged from traces to 3.5 mg% (35 μg/ml), and the average DNOC air concentration in the breathing zone of these workers was 0.0036 mg/liter (3.6 mg/cu m). It was not reported whether there were any signs or symptoms of intoxication.

DNOC accumulated in the blood of five male volunteers who were given 75 mg of DNOC orally for 5 consecutive days [13]. The men experienced an exaggerated sense of well-being when blood levels were about 20 μg/g [17], and headache, lassitude, and malaise were associated with DNOC blood levels of 40-48 μg/g. DNOC was excreted slowly and was still detected in the blood 40 days after the last dose. Another study [21] showed that it took 2-8 weeks for DNOC to be cleared from the serum. In one of the few cases where DNOC in a patient's blood was monitored throughout his recovery period [19], the severity of the symptoms decreased as blood levels decreased.

Several studies of agricultural sprayers who used DNOC solutions have also ascertained that signs of intoxication are associated with blood DNOC levels greater than 20 μg/g [17,19-21]. Exposure was primarily by inhalation, but contact with the skin could not be ruled out. In those studies that allowed comparison of effects in several people, the authors

found that the most severely poisoned individuals of the group had higher blood DNOC levels than their less affected coworkers [17,20]. Workers who exhibited symptoms of intoxication and required hospitalization generally had blood DNOC levels of 20 μg/g or more [17,19-21]. In the reports found, the lowest blood DNOC level in an individual who died was 75 μg/g [17].

Most workers who had blood DNOC levels of less than 10 μg/g were not adversely affected [17,22,27]. There were a few exceptions [20,22,23], but in two of these cases, the investigators [20,22] considered the effects to be only mild. They reported that blood DNOC levels greater than 20 μg/g occurred with severe poisoning.

The data on blood DNOC levels in humans and accompanying effects are summarized in Table III-2. They indicate that workers with DNOC concentrations of 40 μg/g of whole blood (approximately 80 μg/ml of serum) or greater will most likely develop toxic effects. In addition, for the concentration range between 20 and 40 μg/g of whole blood, probably because of variation in individual susceptibility, some workers are affected and others show no adverse effects. Most workers with blood DNOC levels below 20 μg/g were not affected, although, again because of individual susceptibility, some exhibited mild effects.

The effects of DNOC exposure on humans and animals are summarized in Tables III-4 and III-5. In summary, the major effect of exposure to DNOC is a severely increased metabolic rate. Signs and symptoms of intoxication that accompany this effect include an exaggerated sense of well-being, an elevated BMR, profuse sweating, thirst, headache, malaise, and a sensation of heat. Other important effects observed in workers exposed to DNOC are nervous system and gastrointestinal disturbances and kidney damage.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

No studies on the carcinogenic or teratogenic potential of DNOC were found in the literature.

The mutagenic potential of DNOC was tested in four different microbial test systems [40], and the mutation frequency rates were no higher than spontaneous rates. However, because of the previously mentioned inadequacies in the testing procedure, this report cannot be relied upon as conclusive evidence that DNOC is not mutagenic.

Vashakidze [37] investigated the effects of DNOC on the rat reproductive cycle. Although some unspecified effects on the reproductive glands and on the estrous cycle were reported, several shortcomings in the experimental design and presentation of the data prevent adequate assessment of the findings.

TABLE III-1

EFFECTS OF ENVIRONMENTAL TEMPERATURE
ON DNOC TOXICITY IN ANIMALS

Route of Exposure	Species	Dose* (mg/kg)	Temperature	Mortality (%)	Reference
Oral	Rat	40	20-22 C	16.7	29
"	"	40	37-40 C	100	29
"	"	20	20-22 C	0	29
"	"	20	37-40 C	50	29
Subcutaneous	"	27.7	5-10 C	50	33
"	"	24.8	18-20 C	50	33
"	"	19.2	36-37 C	50	33
"	"	20 x36-45 d	cool	8.5	33
"	"	20 x20 d	"	6.5	33
"	"	20 x10 d	"	3.6	33
"	"	20 x17 d	warm	31	33
				LT50**	
"	Mouse	20	10 C	59.0 min	36
"	"	20	20 C	51.3 min	36
"	"	20	40 C	18.8 min	36

*Single dose unless specified
**Time to death of 50% of animals

TABLE III-2

RELATIONSHIP OF BLOOD DNOC LEVELS AND EFFECTS IN HUMANS

Route of Exposure	No.	Occupation	Concentration	Time of Measurement*	Blood DNOC Level (μg/g)	Effects	Reference
Inhalation Dermal	1	Agricultural worker	"Dense mist"	33 hr	1,000**S	Death	21
"	1	"	-	1 wk	200**S	Sweating, labored breathing, vomiting	
"	1	"	-	-	75	Death	17
"	1	"	-	52 hr 7 d	60 25	Headache, lassitude, BMR 275% at 52 hr	19
"	1	"	-	-	60**S	Sweating, headache, labored breathing, fatigue	21
"	1	"	-	24 hr	55	Unconsciousness	20
"	2	"	-	-	44-55	Acute poisoning	17
Oral	5	Experimental subjects	1.0-1.3 mg/kg/d x 5 d	120 hr	40-48	Headache, lassitude, malaise	13
Inhalation Dermal	4	Agricultural workers	-	24 hr	20-40**	Liver damage	20
"	5	"	-	-	30-40	No effects	17
"	6	"	-	24 hr	21-40**	Moderate poisoning; recovery period longer than 8 d	20
"	32	"	-	"	7-37**	Mild poisoning; recovery within 8 d	20
"	1	"	-	"	30**	Fever	20
"	16	"	-	-	20-30	No effects	17
"	1	"	-	24 hr	25**	Kidney damage	20

TABLE III-2 (CONTINUED)

RELATIONSHIP OF BLOOD DNOC LEVELS AND EFFECTS IN HUMANS

Route of Exposure	No.	Occupation	Concentration	Time of Measurement*	Blood DNOC Level (μg/g)	Effects	Reference
Oral	5	Experimental subjects	1.0-1.3 mg/kg/d	96 hr	20	Exaggerated feeling of well-being	13
Inhalation Dermal	21	Agricultural workers	-	-	10-20	No effects	17
"	2	Manufacturing workers	20% in oil	1 wk	8-17	Paresthesia	23
"	149	Agricultural workers	-	-	<10	No effects	17
"	4	"	-	24 hr	4-9**S	Sweating, thirst	22
"	23	"	-	24 hr	1-8**S	No effects	22
"	1	"	-	3 wk	<5**S	Fatigue	21
"	-	"	-	24 hr	1-4	No effects	27

*Time from end of exposure to blood DNOC determinations
**Reported as μg/ml
S indicates serum or plasma DNOC level

TABLE III-3

RELATIONSHIP OF BLOOD DNOC LEVELS AND EFFECTS IN ANIMALS

Route of Exposure	No.	Species	Dose or Concentration	Exposure Duration	Time of Measurement and Blood DNOC Level	Effects	Reference
Inhalation	6	Rats	100 mg/cu m	4 hr	-3 -1 0* 20 hr 4-50 9-42 6-64 17-29 g/ml	Increased respiration	29
"	12	Cats	"	"	0 hr 60-15 g/ml	Death of 4; blood and autonomic nervous system changes	25
"	3	"	60 mg/cu m	"	24 hr 100-150 g/ml	No deaths	25
"	3	"	40 mg/cu m	"	0 hr 40 g/ml	Death of 1; blood and autonomic nervous system changes	25
"	3	"	1.4 mg/cu m	"	Time not reported; No DNOC detected	No deaths	25
"	3	"	2 mg/cu m	4 hr/d x 30 d	" "	Death of 2	25
"	3	"	0.2 mg/cu m	4 hr/d x 2-3 mon	" 10-20 g/ml	No deaths; transient blood changes	25
Subcutaneous	-				0 1 2 3 4 5 6 d		38
		Rats	10 mg/kg	1 dose	82 36 8 3 T - - g/ml	Not reported	
"	-	Cats	"	"	80 36 18 8 3 - - g/ml	"	
"	-	Dogs	"	"	56 40 20 - 3 - T g/ml	"	
"	-	Rabbits	"	"	73 T - - - - - g/ml	"	
"	-	Rabbits	"	1 dose	1 3 5 24 hr 2-25 50-90 25-60 0-8 g/ml	"	

*Immediately after exposure

TABLE III-4

EFFECTS OF OCCUPATIONAL EXPOSURE TO DNOC

Occupation (Mean Concentration DNOC in Air)	Exposure Duration	Exposed Workers			Effects	Reference
		No.	Age	Sex		
Agriculture (-)	-	2	-	M	Brain hemorrhage, death	14
"	2 d	3	17-21	M	Labored breathing, tachycardia, thirst, sweating, 1 death	24
"	6 wk (summer)	133	-	M	Intoxication in 4, 1 death	17
"	5 wk	1	27	M	Headache, elevated BMR	19
"	up to 4 mon	5	25-40	M	Nausea, thirst, sweating, 1 death	21
"	1 d	47	15-44	F	Fever, unconsciousness, liver and kidney damage	20
Agriculture (0.7 mg/cu m)	-	-	-	-	Changes in blood and autonomic nervous system	25
Agriculture (3.6 mg/cu m)	-	20	-	-	No effects	26
Agriculture (-)	50 d (winter)	39	-	M	"	17
Manufacturing (-)	5-30 d	27	24-48	M	Elevated BMR, sweating, mucosal irritation	22
Manufacturing (0.9 mg/cu m)	-	-	-	-	Cardiovascular, CNS, and gastrointestinal effects	25
Manufacturing (-)	6 wk-5 yr	23	-	M	No effects	17
Maintenance (-)	17 d-2 mon	2	24,47	M	Paresthesia	23

TABLE III-5

EFFECTS OF DNOC EXPOSURE IN ANIMALS

Route of Exposure	Species	Exposure Concentration	Exposure Duration	Effects	Reference
Inhalation	Rat	100 mg/cu m	4 hr	Decreased respiration	29
"	Cat	100 mg/cu m	"	Death in 4 of 12; autonomic nervous system effects; blood changes	25
"	"	60 mg/cu m	"	No deaths; other effects as at 100 mg/cu m	25
"	"	40 mg/cu m	"	Death in 1 of 3; other effects as at 100 mg/cu m	25
"	"	2.0 mg/cu m	4 hr/d 1 mon	Death in 2 of 3; blood changes, weight loss	25
"	"	1.4 mg/cu m	4 hr	No effects	25
"	"	0.2 mg/cu m	4 hr/d 2-3 mon	No deaths; transient blood changes	25
Dermal	Rat	2%	30 d	No effects	15
"	Mouse	187 mg/kg	1 dose	LD50	31
"	Rabbit	500 mg/kg	"	Changes in respiration and heart rate	26
"	"	500 mg/kg	"	No effects	26
"	"	1,000 mg/kg	"	LD50	26
"	"	2%	30 d	No effects	15
"	Guinea pig	500 mg/kg	1 dose	100% mortality	34
"	"	200 mg/kg	"	No deaths	34

TABLE III-5 (CONTINUED)

EFFECTS OF DNOC EXPOSURE IN ANIMALS

Route of Exposure	Species	Exposure Concentration	Exposure Duration	Effects	Reference
Oral	Rat	85 mg/kg	1 dose	LD50	26
"	"	40 mg/kg	"	Death in 2 of 12	29
"	"	30 mg/kg	"	Minimum lethal dose	15
"	"	20 mg/kg	"	No effects	29
"	"	0.005 mg/kg/d	2 mon	"	30
"	Mouse	47 mg/kg	1 dose	LD50	26
"	"	16.4 mg/kg	"	"	31
"	Hare	24.8 mg/kg	"	"	32
"	Cat	50 mg/kg	"	"	26
"	Pheasant	8.4 mg/kg	"	"	32
"	"	7.1 mg/kg/d	3 d	"	32
"	Partridge	11.1 mg/kg/d	"	"	32
"	"	8.3 mg/kg	1 dose	"	32
Sub-cutaneous	Rat	26-39 mg/kg	"	"	35
"	"	20 mg/kg	"	Minimum lethal dose	15
"	"	15 mg/kg/d	30 d	Slight weight gain	15
"	Mouse	24.2 mg/kg	1 dose	LD50	33