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Review

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Toxicity and Metabolism of Nitroalkanes and Substituted Nitroalkanes

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ABSTRACT: A series of low molecular weight nitro-containing compounds has recently been discovered to have a variety of biological activities including the reduction of anaerobic methane production in ruminant animals and activity against economically important human pathogens, including *Salmonella* sp. and shigella-toxin producing *Escherichia coli*. Although some of these nitrocompounds, nitroethane and 2-nitropropane, for example, have been industrial chemicals and synthetic intermediates for years, others such as carboxymethyl nitro−amino acid analogues are new to science and have not been previously described. The purpose of this paper is to review the toxicological profiles, especially as related to events occurring during metabolism and biotransformation, which contribute to toxicological end points of established nitroaliphatic compounds. It is hoped that by summarizing existing knowledge, an understanding of the activities and toxicological profiles of newly established nitrocompounds might be anticipated or adverse events associated with their use might be avoided.

KEYWORDS: *nitroalkanes, toxicity, metabolism*

INTRODUCTION

A class of novel, nitrosubstituted structural analogues of 3-nitropropanol (a plant metabolite) has been discovered to be potent inhibitors of *Salmonella*, *Escherichia coli* O157:H7,[1,2] and *Camplobacter*.[3] In addition, a number of the nitrocompounds have been shown to inhibit both in vitro[4−6] and in vivo[1] ruminal methane production. Because eructation of methane represents from 2 to 12% of a ruminant's gross energy intake,[7] a reduction in the fraction of feed energy converted to methane without concomitant perturbation of ruminal fermentation would have far-reaching implications in global food production. In addition, reduction in methane production at municipal waste-processing plants could decrease the carbon footprint associated with sewage treatment.

Although newly discovered, use of nitrocompounds for the elimination of pathogens and (or) the inhibition of methanogenesis has the potential to greatly benefit animal producers, the public, and the environment. However, little to no research has been conducted to demonstrate the safety and fate of these novel nitrocompounds in either animals or the environment, and virtually no data exist for many that will determine their viability as products with commercial application. Nevertheless, a body of literature does exist that summarizes the toxicity and metabolic fate of a diverse group of manmade and naturally occurring short-chain nitroalkanes, nitroalcohols, and nitroacids. It is the purpose of this review to summarize the existing literature on the toxicity of known nitrocompounds, especially in the context of the metabolic events that occur during their absorption, distribution, metabolism, and excretion.

General Chemistry of Nitrocompounds. Nitroalkanes (R—NO_2) should be distinguished from nitroesters of aliphatic alcohols (R—ONO_2) even though both are sometimes referenced as "nitro" compounds in the open literature. In addition, alkyl nitrites (R—O—N═O), which are structural isomers of nitroalkanes, are chemically distinct. Nitroalkanes and nitroesters have unique synthetic pathways and are characterized by unique chemical and biological properties. For example, nitroalkanes are typically formed by the reaction of alkyl halides with nitrites, the vapor phase nitration of hydrocarbons, the oxidation of oximes with halogenated peroxyacids, or the oxidation of carbinamines. In contrast, nitroesters are typically prepared by the simple esterification of alcohols with nitrous (HNO_2) or nitric (HNO_3) acids. Whereas the stability of nitroalkanes is inversely proportional to the aliphatic chain length,[8] nitroesters are labile compounds and must be handled appropriately.

Nitro groups present on nitroaliphatics have an electron-withdrawing influence on the carbon to which they are bonded (the α-carbon) and confer acidity to the α-carbon. α-Carbon acidity of nitroalkanes (pK_a of 4−6) contributes to their reactivity and also their biological activity at the enzyme level. Nitronic acids (Scheme 1) can be produced by reaction of nitroalkanes with alkali followed by reacidification wherein *aci*

Scheme 1

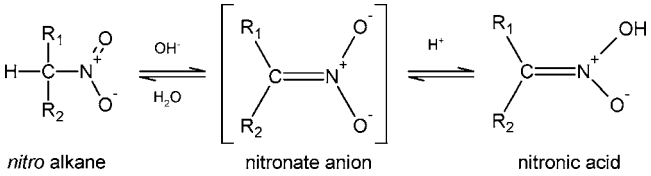

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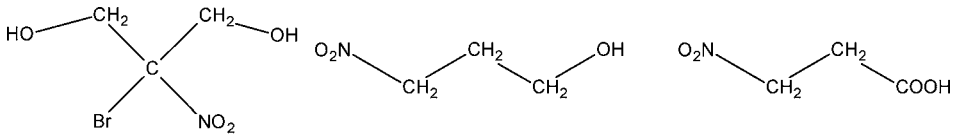
Table 1. Use of Nitroalkane and Nitroalkane Derivatives in Industrial Applications[a]

	use										
application	chemical synthesis	pH control	corrosion control	cross-linking	dispersion	emulsification	moisture control	oxygen scavenging	radical scavenging	solvent	fuel
ag chemicals	Y										
biocides	Y										
hobby/racing											Y
inks		Y	Y		Y					Y	
leather				Y							
life sciences		Y									
metalworking	Y	Y	Y			Y					
mineral slurries		Y			Y						
paints and coatings		Y	Y		Y	Y	Y			Y	
personal care		Y	Y		Y	Y					
pharmaceuticals	Y	Y									
solvents/thinners										Y	
synthesis	Y									Y	
synthetic rubber								Y	Y		
water treatment		Y						Y			
wood adhesives											

[a]Adapted from *Putting Nitroalkane Chemistry to Work for You*, Angus Chemical Product Guide, Angus Chemical Co., Buffalo Grove, IL, USA.



1 2 3

Figure 1. Structures of Bronopol (**1**), 3-nitropropanol (**2**), and 3-nitropropionic acid (**3**).

isomers are formed by the facile protonation of the nitronate anion intermediate (Scheme 1). In reality, *aci* and parent nitroalkanes are tautomers that can be isolated.[8,9] The ability to form stable tautomers has important implications for the toxicity of secondary nitroalkanes[10,11] described in more detail below.

The relative stability of nitroalkanes, the ability to manipulate the reactivity of the α-carbon, and the versatility of the nitro functional group have rendered nitroalkanes useful as intermediates in a multitude of chemical syntheses having widespread application in industrial processes.[8,12] As might be expected, modification of carbons adjacent to nitrocarbons will influence the α-carbon reactivity. For example, addition of electron-withdrawing groups on adjacent carbons of nitroalkanes tends to increase α-carbon acidity, and substitution of the α-hydrogen with an electron-rich leaving group (such as a halogen) will potentiate reactivity toward electrophiles. Such modifications have been exploited with the development of 2-bromo-2-nitro-1,3-propanediol (**1**) (Bronopol), a widely used antimicrobial preservative[13] described in more detail below.

OCCURRENCES AND USES OF NITROALKANES AND SUBSTITUTED NITROALKANES

Aliphatic Nitroalkanes. Unsubstituted nitroalkanes are not generally thought of as being produced by biological processes and, to the authors' knowledge, there are no biological sources of aliphatic nitroalkanes. Although not technically produced by nature, short-chain nitroalkanes (one to five carbons) are present at low levels in cigarette smoke,[14] and human exposures may occur through this route. More importantly for human exposures, nitroalkanes are used industrially for a multitude of purposes (Table 1) including chemical intermediates for agrochemical and pharmaceutical syntheses, as solvents for inks, resins, paints, and coatings, as cross-linking agents for leather tanning, for stabilization of halogenated hydrocarbons, and as cleaning solvents for circuit boards. Major nitroalkanes used for commercial applications include nitromethane, nitroethane, 1-nitropropane, and 2-nitropropane.[8] The body of literature available on nitroalkane toxicity, metabolism, and physiological disposition was generated mostly in response to occupational exposures through industrial nitroalkane applications.

Aliphatic Nitroalcohols and Nitroacids. Broadly defined, aliphatic nitroalcohols are straight- or branch-chained carbon backbones having one or more nitro and alcohol functional groups. Aliphatic nitroalcohols are commercially important, and their synthesis and reactivities have been intensively studied.[15] Typically, carbon chain length for commercially important nitroalcohols does not exceed five carbons.[15] The most important commercial nitroalcohol for life-science applications is 2-bromo-2-nitro-1,3-propanediol (**1**; Bronopol) (Figure 1), which has been used as an antibacterial agent in cosmetics, personal care products, pharmaceuticals, water treatment plants, and other industrial processes since the mid-1960s.[13]

Structure−activity studies with Bronopol analogues indicate that 1-nitroalkanes possess little antimicrobial activity; activity against the microbes and fungi tested almost always required a $Br-C(R)_2-NO_2$ structure. Unhalogenated nitroalcohols possessed relatively low activity, and substitution of bromine with chlorine also reduced activity. Introduction of an alcohol adjacent to the bromo-nitrocarbon enhanced activity significantly.[16] The activity of Bronopol is believed to be due to the

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facile formation of an electrophilic carbocation at C-2 and its subsequent ability to disrupt cellular proteins and components after nucleophilic binding. As discussed below, this reactivity, which is beneficial for use as a preservative, also carries some negative toxicological implications for human exposures.

In some rangeland plants, especially *Astragalus*, *Coronilla*, and *Indigofera* species, naturally occurring 3-nitropropanol (**2**) and 3-nitropropionic acid (**3**) (Figure 1) are present as mono-, di-, or triglycoside conjugates[17,18] or as unconjugated species. 3-Nitropopanol and 3-nitropropionic acid and their respective glycosides or glucose esters are not present together within a plant species. The *O*-glycoside(s) of nitropropanol, collectively known as *miserotoxins*, have gained some degree of notoriety with western livestock producers[18,19] due to their toxicities under certain grazing circumstances. Although glucose conjugates of 3-nitropropionic acids contribute to a greater or equal extent to animal morbidity and mortality as 3-nitropropanol glycosides, they are only generally classified as "nitrotoxins".

Human exposure to 3-nitropropionic acid may occur through the consumption of foods fermented with *Aspergillus* and *Penicillium* species or through foods contaminated with fungal species (reviewed by Burdock et al.[20]). Due to the chronic nature of these exposures and the large populations of exposed individuals, acceptable daily intakes have been estimated to be 25 μg/kg body weight per day.[20] In contrast to 3-nitropropanol, 3-nitropropionic acid has attracted a notable amount of attention within the biomedical community due to its usefulness as a model for Huntington's disease.[21,22]

TOXICITIES

Description of Animal and Human Toxicity. Generally stated, nitroalkanes, nitroalcohols, and nitroacids are well tolerated in mammalian systems. During acute, high-level exposures, denitrification may release sufficient nitrite to cause classical nitrite-induced methemogloninemia. Typically, the methemogobinemia is transient and causes no lasting effects. Toxicological end points associated with chronic exposures to low levels of nitrocompounds have typically been of greater concern than acute exposures. Although compound specific, the most important toxicological end points are typically mutagenicity and genotoxicity with associated outcomes such as tumorogenicity, teratogenicity, and carcinogenicity. Noteworthy in this regard is the fact that only secondary nitroalkanes, specifically 2-nitropropane, are considered to be potential or suspect carcinogens.

Human and animal exposures to appreciable amounts of the nitroparaffins (nitromethane, nitroethane, 1-nitropropane, and 2-nitropropane) are generally uncommon and occur mainly via inhalation in the workplace.[23] Exposure of the general human population to low quantities of 2-nitropropane (1.1−1.2 μg/cigarette) can occur from inhalation of tobacco smoke.[24] Oral LD_{50} estimates and relevant threshold limit values for select common nitroparaffins are presented in Table 2, and summaries of toxicities for these nitroparaffins can be found on the the National Toxicology Program (NTP) Web site (http://ntp.niehs.nih.gov/). With respect to nitroparaffins, acute and chronic exposures to toxic levels result in liver damage, central nervous system depression, and kidney damage. Irritation of eyes and respiratory tracks can also occur. 2-Nitropropane is reported to be mutagenic and genotoxic.[25] As reported on the NTP Web site, nitromethane and 2-nitropropane are each listed as *reasonably anticipated to be a human carcinogen*,[26] but neither nitroethane nor 1-nitropropane was mutagenic or carcinogenic in the Ames *Salmonella* assay or in a number of cell culture tests. Similarly, 1-nitrobutane, a related compound, returned negative results when tested in the Ames *Salmonella* assay. A rationale for these differences in toxicity is presented during the discussion on biotransformation reactions below.

Table 2. Oral LD_{50} and Maximum Threshold Limits Applications of Nitroparaffins

	LD_{50}[a] (mg/kg BW)				
compound	intraperitoneal/mouse	oral/mouse	oral/rat	dermal/rabbit	threshold limit values[a] (ppm)[b]
nitromethane	110	950	940	7200	20
nitroethane	310	860	1100		100
1-nitropropane	250	800	455	>2000	25
2-nitropropane	800		720	>2000	10

[a]NTP at http://ntp.niehs.nih.gov/. [b]See ref 23.

Human mortalities have occurred following acute inhalation exposure to 2-nitropropane concentrations of up to 600 ppm.[26] The few cases of inhalation exposure of humans to nitromethane, nitroethane, or 1-nitropropane have been less severe, resulting mainly in methemoglobinemia. Accidental ingestion of a model airplane fuel mixture containing methanol and nitromethane also resulted in nonlethal methemoglobinemia. Similarly, ingestion of fingernail polish remover containing nitroethane resulted in delayed onset or recurrent methemoglobinemia, but both cases were successfully treated.[27,28]

In studies with livestock species, overt signs of toxicity were not observed following oral administration of as much as 160 mg nitroethane/kg body weight per day to feedlot steers over 16 consecutive days.[29] Similarly, adverse effects were not observed in adult ewes orally administered as much as 72 mg nitroethane/kg body weight per day over a 5 day period.[1]

Less is known regarding the toxicity of nitroalcohols, although they are reported to present less of an inhalation hazard because of their low volatility.[23] Fridman and colleagues[30] reported that the acute LD_{50} to mice following intraperitoneal injection of 2-nitroethanol was 2100 mg/kg body weight and that toxicities were higher with nitro-substitution or halogen substitution on the α-carbon, with brominated nitroalcohols being most toxic. Acute oral LD_{50} values with mice for 2-nitro-1-butanol, 2-methyl-2-nitro-1-propanol, 2-methyl-2-nitro-1,3-propanediol, 2-ethyl-2-nitro-1,3-propanediol, and 2-hydroxymethyl-2-nitro-1,3-propanediol are reported to be 1200, 1000, 4000, 2800, and 1900 mg/kg body weight, respectively.[31] The acute oral LD_{50} values of 2-bromo-2-nitro-1,3-propanediol (Bronopol) in male and female rats are 307 and 342 mg/kg body weight, respectively.[32] In the case of 2-nitro-1-propanol, Jung et al.[33] reported 0, 10, and 30% mortalities in 6-day-old broiler chicks (10 per treatment) orally administered 87, 433, and 867 mg/kg bw (with the assumption that chicks weighed 0.150 kg at 6 days) 2-nitro-1-propanol per bird, respectively. A daily dose of 120 mg 2-nitro-1-propanol/kg body weight per day was orally administered over a period of five consecutive days to adult ewes with no apparent adverse effect.[1] When tested in the *Salmonella* Ames test, 2-nitroethanol was reported to be positive in one laboratory but results from another laboratory were inconclusive.[34] 2-Nitro-1-butanol was negative[34] in the Ames *Salmonella* test. Equivocal results with 2-nitroethanol were obtained from the micronucleus test with

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male mice.[26] Bromo-2-nitro-1,3-propanediol (Bronopol) was not reported to be carcinogenic or mutagenic.[32]

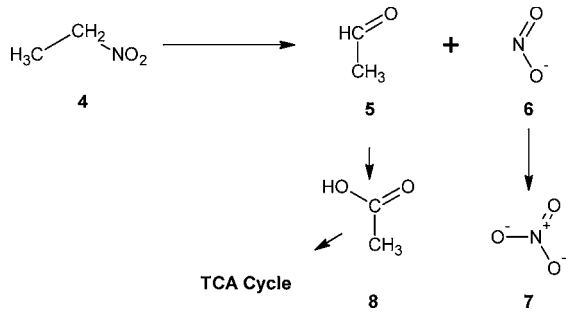
Human exposures to the naturally occurring nitrocompounds 3-nitro-1-propionic acid and 3-nitro-1-propanol occur primarily via ingestion of moldy foods, whereas livestock poisonings by these naturally occurring nitrocompounds occur due to ingestion of nitrosynthesizing forages such as *Coronilla varia*, and certain species of *Astragalus* and *Indigofera*.[18,20] 3-Nitro-1-propionic acid is a suicide inhibitor of succinate dehydrogenase and, thus, toxicity is manifested via inhibition of cellular respiration.[35] 3-Nitro-1-propanol is converted to 3-nitro-1-propionic acid by mammailian hepatic alcohol dehydrogenase, and its toxicity is similarly manifested via inhibition of cellular respiration.[36] The acute intraperitoneal LD_{50} values of 3-nitro-1-propanol and 3-nitro-1-propionic acid in rats are 61 and 67 mg/kg bw, respectively.[37] According to a testing status report at NTP, equivocal evidence of carcinogenicity was obtained following bioassay with male rats orally administered 0.85 mg of 3-nitro-1-propionic acid per animal per day for 110 weeks. However, no evidence of carcinogenicity was observed in female rats similarly administered 0.6 or 1.2 mg of 3-nitro-1-propionic acid or in male or female mice orally administered 0.375 or 0.75 mg of 3-nitro-1-propionic acid per day.

Absorption, Distribution, Metabolism, and Excretion (ADME). Nitrocompounds are not generally caustic and do not generally have significant contact toxicities, with the exception of Bronopol (discussed below). Toxicity generated from nitrocompound exposure is typically mediated after systemic absorption and often occurs only after metabolism of the parent nitrocompound has taken place. Thus, a discussion of absorption, tissue distribution, metabolism, and excretion is highly relevant in the consideration of toxic pathways and mechanisms of nitrocompounds.

Published data on the in vivo ADME of nitroalkanes, nitroalcohols, or nitroamino acids in mammalian or avian species are few considering the length of time that these compounds have had global economic impacts. Most data are available for nitroalkanes used in industrial processes and for which occupational exposures might occur. Worldwide use of Bronopol in cosmetic and personal care products has resulted in the availability of some ADME data, but considering the volume of Bronopol use, these studies are relatively sparse. Finally, a few studies have been published on nitroalcohols and nitroacids of toxicological concern, primarily to livestock producers. Due to expenses associated with radiolabeled studies in livestock, these studies have exclusively employed nonlabeled test articles, so the overall fate and disposition of the natural nitroalcohols and ntiroacids have not been well described. An additional body of literature across all compounds describes in vitro work in which the transformation of nitro compounds in specific tissues or cell types or with specific enzymes was investigated with the hopes of drawing associations with, or correlations to, toxicity.

ADME of Nitroethane. Using massive doses (300−1000 mg/kg bw) of nitroethane (**4**), and within the analytical limitations of the time, Machle et al.[38] showed that in rabbits, only 17−19% of oral or intravenous doses could be recovered as the parent nitroethane. These results strongly suggested that biotransformation processes occurred rapidly and were responsible for the major portion of dosed nitroalkane. Scott[39] determined that acetaldehyde (**5**) and nitrite (**6**) were formed in blood in both nitroethane-dosed animals and in control blood fortified with nitroethane. Although nitrite was formed and was present in the blood of dosed animals, it represented only a fraction of the dosed nitroethane nitrogen. Nitrite concentrations decreased rapidly, presumably due to reaction with hemoglobin. Subsequent study by Scott[40] confirmed oxidation of nitrite to nitrate (**7**) in nitroethane-dosed animals and defined the metabolism of nitroethane as that shown in Scheme 2.

Scheme 2. Biotransformation of Nitroethane (Based on References 39 and 40)



Proposals for the fate of acetaldehyde (**5**) were not made by Scott,[39] but later work demonstrated that acetaldehyde is oxidized by acetaldehyde dehydrognease to acetate (**8**), a substrate for the tricarboxylic acid cycle.[41] Nitrite formation was short-lived due to its oxidation during the reduction of hemoglobin to methemoglobin. The formation of methemoglobin during nitroethane intoxication was verified by Matsumoto et al.[42] in 1961. Thus, the metabolism of nitroethane to release nitrite is a critical factor in its acute toxicity.

Although nitroethane has been a fairly common industrial solvent, human exposures through oral routes were very unlikely until fairly recently. In the past 20 years, nitroethane has become the main solvent for some artificial fingernail removal products. Home use of such solvents has increased the opportunity for nitroethane ingestion by children, and several poisonings have occurred,[27,28] with methemoglobinemia being the major clinical finding. With regard to industrial uses where inhalation is the likeliest exposure route, Stott and McKenna[43] showed that both the upper and lower respiratory tracts of rats have high capacities to absorb nitroethane. For example, 65−71% of a 1000 ppm nitroethane dose dissolved in air during a 2 h period was absorbed. Although the lung did have the capacity to excrete nitroethane, only a small portion of the absorbed fraction was eliminated via the lung. These data are consistent with the early findings of Machle et al.[38]

The enzymology of nitroethane transformation in animals has not been investigated, although a large body of literature exists on nitroalkane oxidase and nitronate monooxygenase, enzymes capable of transforming primary and secondary nitroalkanes in fungi, yeasts, and bacteria. Nitroalkane oxygenase is a fungal enzyme isolated from *Fusarium oxysporum*, which catalyzes the oxidation of primary and secondary nitroalkanes according to Scheme 3: The substrate preference for nitroalkane oxidase is un-ionized primary nitroalkanes.[44] This differs appreciably from yeast and bacterial nitronate monooxygenases, which preferentially oxidize secondary anionic nitroalkanes[45] according to Scheme 4.

ADME of 1-Nitropropane. A single radiolabeled metabolism study describes the metabolism and disposition of 1-nitro-

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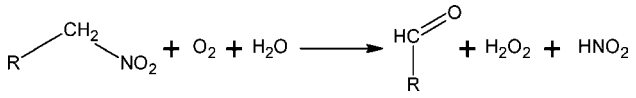
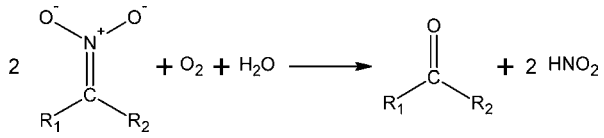
Scheme 3. Action of Nitroalkane Oxygenase on a Primary Nitroalkane



Scheme 4. Action of Nitronate Monooxygenase on Secondary Nitroalkanes



propane (**9**) in rats and chimpanzees.[46] Rats were dosed with 40 mg/kg bw of 1-nitro[1-^{14}C]propane as a single intraperitoneal injection, and groups of animals were killed after a 12 or 48 h period. Urine and feces were collected from all of the animals, and expiratory gases were collected from the rats slaughtered at 48 h. Gases were sampled so that loss of parent 1-nitropropane through respiration could be measured as well as the losses of ^{14}C-CO_2 through complete oxidation of the dosed material. Chimpanzees were dosed with 5 mg/kg bw of 1-nitro[1-^{14}C]propane via intravenous injection, and urine and feces were collected for 96 h; respiratory gases and tissus were not collected from chimpanzees.

The disposition of radioactivity dosed as 1-nitro[1-^{14}C]-propane to rats and chimpanzees is shown in Table 3.

Table 3. Disposition of Radioactivity in Rats and Chimpanzees Administered a Single 1-Nitropropane Dose[46]

fraction	rat[a] (% of dose)	chimpanzee[b] (% of dose)
air	72.6	not measured
carcass	6.5	not measured
feces	1.8	2.2
urine	16.5	15.1
total	97.4	17.3

[a]A single 40 mg/kg bw dose was administered via intraperitoneal injection. Air, urine, and fecal data are cumulative to 48 h. Carcass data were collected at 48 h. [b]A single 5 mg/kg bw dose was administered via intravenous injection. Urine and fecal data are cumulative to 98 h.

Cumulative urinary and fecal excretions were consistent between species with roughly 15% of the dose excreted in urine and 2% excreted in feces. Low levels of radioactivity in fecal matter of both species suggest that biliary elimination of 1-nitropropane and (or) its metabolites represent a minor route of elimination. By far, the major route of elimination in rats was through respiratory losses, representing nearly 75% of the dosed radioactivity. A similar respiratory loss in chimpanzees is implied by comparable recoveries of radioactivity in urine and feces of both rats and chimpanzees. In rats, 14.2% of the expired radioactivity was identified as parent 1-nitropropane, representing 10.3% of the total radioactive dose; the remaining respiratory radioactivity, representing 62.3% of the dose, was trapped as ^{14}C-CO_2. The compositions of urinary radioactivity from both rats and chimpanzees were qualitatively similar, with very polar metabolites being excreted by both species. Two major metabolites (3-hydroxypropionic acid (**10**) and *N*-methyl-*N*-2-(methylsulfinyl)ethyl propionic acid amide, NMPA (**11**)) were identified (Scheme 5) using ^{1}H and ^{13}C NMR, mass spectrometry, and synthesis of authentic standards. Three minor metabolites were not identified, and propionaldehyde (not shown) was not detected. Haas-Jobelius et al.[46] proposed the formation of the unusual hydroxyacid and NMPA metabolites due to saturation of the normal metabolic pathways of propionyl coenzyme A. The initial step of 1-nitropropane metabolism in animals is denitrification, which likely occurs through cytochrome P450 mediated reactions.[47,48] Mechanisms of 1-nitropropane denitrification have not been studied in depth in higher organisms.

ADME of 2-Nitropropane. Unlike 1-nitropropane, a great deal of research has been conducted on the metabolism 2-nitropropane (**12**) (Scheme 6), particularly with regard to elucidating its mechanism of genotoxicity. Even with the large body of literature, few whole-animal studies have been conducted, and only two radiolabeled studies in live animals have been published. The first of these was a 1982 study by Nolan et al.,[49] who investigated the kinetics of [1,3-^{14}C]-2-nitropropane depuration in rats after 6 h inhalation exposures to 20 or 154 ppm of 2-NPA vapor in air. Elimination of radioactivity was measured during a 48 h period, and body distribution was determined at euthanasia (48 h; Table 4). The authors estimated that at least 40% of the inhaled 2-nitropropane dosed to rats was absorbed by the lung. Similar to 1-nitropropane, once absorbed, the major route for elimination was respiratory loss, regardless of dose. The major respiratory fraction (52% of recovered radiocarbon) was $^{14}CO_2$, indicating that 2-nitropropane is metabolized to products capable of entering intermediary metabolism. The relatively large portion (25% of recovered radiocarbon) of tissue and carcass radioactivity also suggested a major incorporation of ^{14}C-carbon into natural products of intermediary metabolism. Rats exposed to the higher concentration of 2-nitropropane eliminated 50 times the amount of 2-nitropropane from the lungs during the first depuration hour than did rats in the low-exposure group. Regardless of exposure, 87% of the total $^{14}CO_2$ eliminated was expired during the initial 12 h of the study period. Blood radioactivity was not proportional to dose, indicating that dose-dependent kinetics of 2-nitropropane occurs; the half-life of parent 2-nitropropane in the blood could not be estimated for the low-dose group, but was estimated to be only 48 min in the high-dose rats. In contrast, α-(distribution) and β- (elimination) half-lives of radioactivity (parent and metabolites) were 1.1 and 16.4 h, respectively, in the low-exposure group and 1.2 and 13.2 h, respectively, in the high-exposure group. Radioactive residues in tissues (48 h) tended to be greatest in organs involved with residue elimination, that is, the lungs, kidney, and liver. The authors did not consider alkylation (covalent binding) to tissue macromolecules a likely cause of toxicity, but rather that radioactive residues in tissues were present as normal products of metabolism. Nolan et al.[49] did not report identities of metabolites in tissues or excreta.

Müller et al.[50] conducted a series of studies in rats with ^{14}C-2-nitropropane using inhalation (200 ppm for 3 h) and intraperitoneal (25 and 50 mg/kg bw) exposures; chimpanzees were also exposed to intravenous ^{14}C-2-nitropropane (10 mg/kg bw) to determine its metabolism in nonhuman primates. The position of the radiocarbon in the 2-nitropropane was not provided. Gross disposition, shown in Table 5, was similar to results obtained by Nolan et al.[49] in that respiratory loss of radioactivity was the major route of excretion, with urinary and

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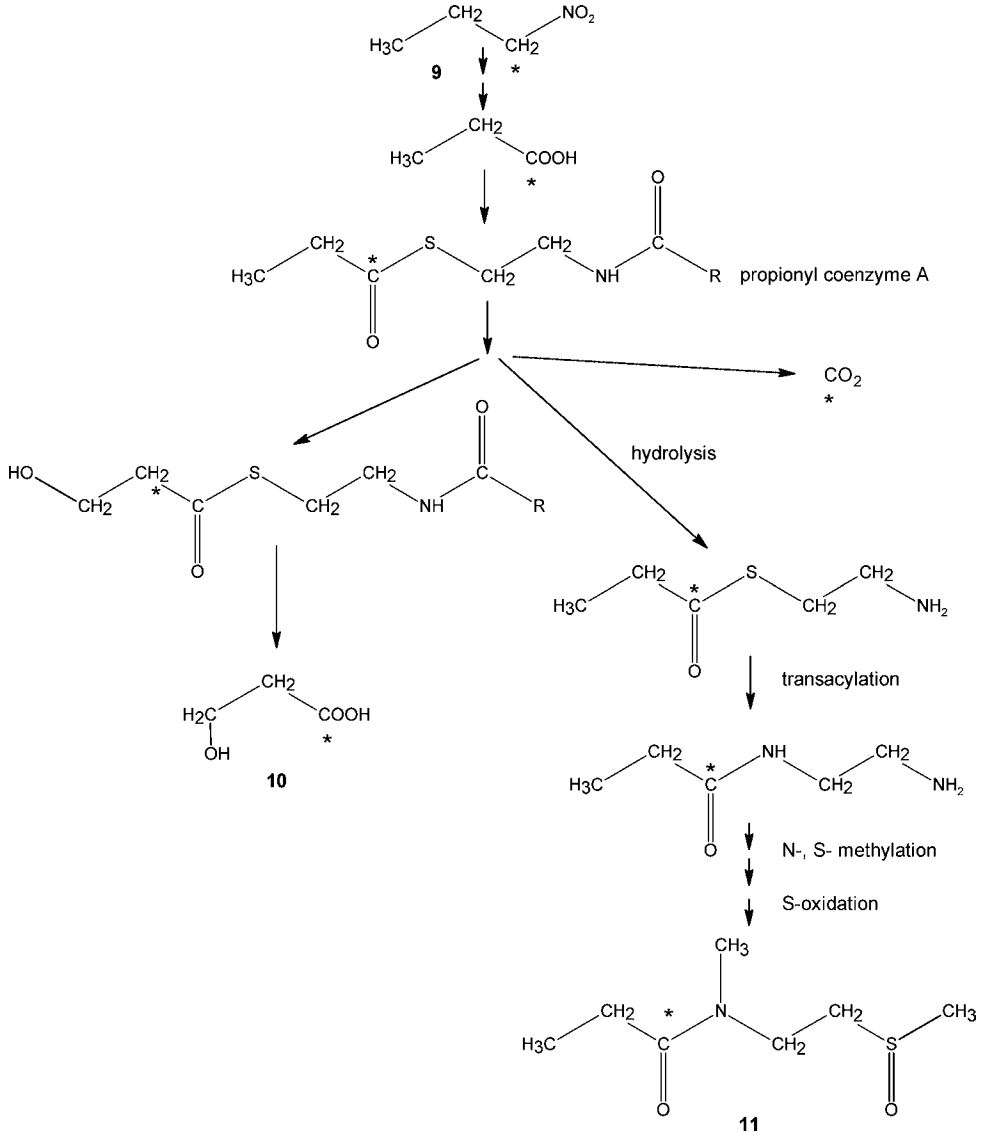
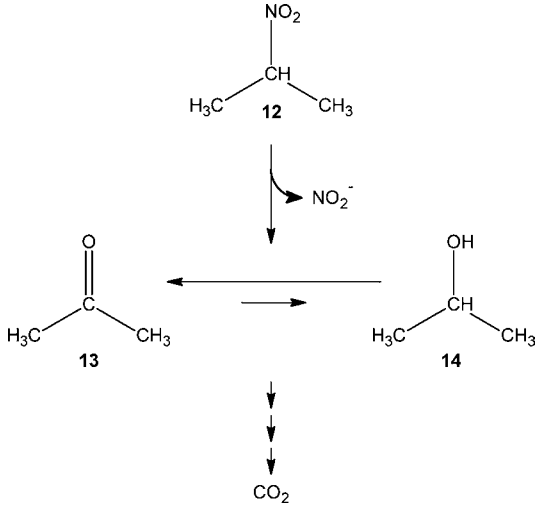
Scheme 5. Biotransformation of 1-Nitropropane in Rats and Chimpanzees[a]



[a]Asterisks indicate the location of radiocarbon label (adapted from ref 46).

Scheme 6. Biotransformation of 2-Nitropropane in Rats and Chimpanzees



fecal excretion being minor components. Tissue accumulations of radioactivity at 1, 40, and 192 h after a single intraperitoneal dose with 50 mg/kg bw 2-nitropropane were unremarkable with the exception of the accumulation of radioactivity in the adrenal gland and spleen, a finding that the authors hypothesized was related to the incorporation of radioactivity into steroids via the mevalonate pathway.

Müller et al.[50] demonstrated that respiratory radioactivity consisted of acetone (**13**) as a major metabolite, parent 2-nitropropane (**12**) as a minor component, and $^{14}CO_2$ (Table 5). Urinary metabolites at 6 and 40 h postdosing consisted of a major unidentified metabolite (60−80% of radioactivity), isopropanol (**14**) (8−18%), acetone (**13**) (10−12%), and 2-nitropropane (**12**) (0−12%) (Scheme 6).

Although no isopropyl alcohol was measured in expired air, it was clearly present as a metabolite in urine and blood. The kinetics of the appearance of acetone and 2-propanol and the disappearance of 2-nitropropane (Figure 2) suggest that denitrification of 2-nitropropane is the major metabolic route of 2-nitropropane in rats. Both acetone and isopropyl alcohol

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Table 4. Disposition of Radioactivity in Rats after Inhalation Exposure to 20 or 154 ppm of [1,3-^{14}C]-2-Nitropropane for 6 h[49a]

fraction	20 ppm exposure		154 ppm exposure	
	mg equiv[b]	%[c]	mg equiv[b]	%[c]
expired air				
^{14}C-CO_2	0.558 ± 0.117	51.9 ± 7.2	6.44 ± 0.82	50.8 ± 2.0
2-nitropropane	0.040 ± 0.005	3.7 ± 0.2	2.75 ± 0.38	21.9 ± 4.6
urine	0.087 ± 0.010	8.1 ± 0.7	1.36 ± 0.23	10.7 ± 0.9
feces	0.111 ± 0.048	10.7 ± 5.6	0.68 ± 0.28	5.3 ± 1.6
tissues and carcass	0.270 ± 0.019	25.5 ± 2.3	1.43 ± 0.15	11.3 ± 0.9
total	1.066 ± 0.087		12.66 ± 1.09	

[a]Air, urine, and fecal data are cumulative to 48 h. Carcass data were collected at 48 h. [b]Radioactive residues are expressed as 2-nitropropane equivalents. [c]Data are expressed as percentages of recovered radioactivity.

Table 5. Disposition of Radioactivity in Rats and Chimpanzees Administered a Single Dose of ^{14}C-2-Nitropropane[50]

fraction	rat[a] (% of dose)	chimpanzee[b] (% of dose)
air		
2-nitropropane	4.5	not determined
acetone	10.4	not determined
CO_2	38.1	not determined
carcass	12.0	not determined
feces	0.7	0.5
urine	5.9	5.6
total	71.6	

[a]A single 50 mg/kg bw dose was administered via intraperitoneal injection. Air, urine, and fecal data are cumulative to 40 h. Carcass data were collected at 48 h. [b]A single 5 mg/kg bw dose was administered via intravenous injection. Urine and fecal data are cumulative to 98 h.

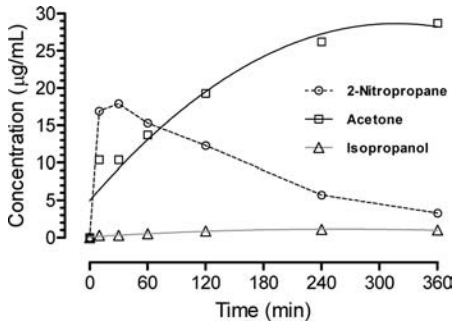

Figure 2. Blood levels of 2-nitroproane, isopropanol, and acetone in rats dosed intraperitoneally with 40 mg/kg bw of 2-nitropropane (adapted from ref 50).

are oxidized to CO_2 in rats,[51,52] which is likely their fate after formation from 2-nitropropanol. The study of Müller et al.[50] is the only study to measure isopropanol as a major product of 2-nitropropane metabolism.

Oxidative denitrification of 2-nitropropane to acetone and nitrite occurs in rat liver microsomal fractions via cytochrome P450 catalyzed reactions. Animals pretreated with phenobarbital and 3-methylcholanthrene, nonspecific inducers of cytochrome P450 expression, had microsomes with increased denitrification activity; addition of cytochrome P450 inhibitors such as metyrapone, carbon monoxide, and benzflavone reduced dentirification activities. Cytochrome P450 mediated oxidative denitrification was proposed to occur through the formation of a 2-hydroxy-2-nitropropane intermediate,[47] a reaction mechanism supported by the microsomal cumene peroxide denitrificaion of 2-nitropropane.[53]

The involvement of enzymatic metabolism with 2-nitropropane toxicity was suggested by data of Roscher et al.,[54] who demonstrated that induction of DNA repair, micronuclei, and 6-thioguanine resistance—end points of genotoxicity—occurred for 2-nitropropane only in cells capable of expressing cytochrome P450s. Later studies determined that rabbit cytochrome P450F1, a member of the phenobarbital-inducible P450s, was capable of denitrifying both 1- and 2-nitropropane.[48] Cytochrome P450s were capable of denitrifying both primary and secondary nitroalkanes, but not tertiary nitroalkanes, indicating a requirement for α-hydrogen abstraction[55] in the enzyme-mediated reaction. However, in 1998, it was demonstrated that cytochrome P450 denitrifying activity was not necessarily related to 2-nitroalkane toxicity, especially 2-nitroalkane genotoxicity.[56] For example, treatment of ovine seminal vesicle cells, a tissue devoid of cytochrome P450 monooxygenase activity, with 2-nitropropane or propane-2-nitronate caused significant induction of DNA repair mechanisms and the formation of specific DNA modifications. The authors demonstrated that genotoxicity of 2-nitropropane was mediated through the actions of seminal vesicle sulfotransferase activity (discussed below).

Reductive metabolism of 2-nitropropane has also been investigated for possible involvement in the genotoxicity of 2-nitropropane even though reductive metabolites were not measured during live-animal studies. Specifically, the formation of acetone oxime (**15**; Figure 3) was measured in cultured rat

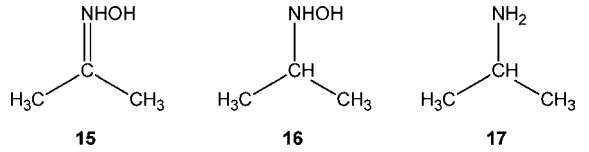

Figure 3. Structures of acetone oxime (**15**), isopropyl hydroxylamine (**16**), and 2-aminopropane (**17**).

hepatocytes and in Chinese hamster ovary cells.[57] Neither acetone oxime (**15**) nor the hypothetical reduction products isopropyl hydroxylamine (**16**) or 2-aminopropane (**17**) were able to replicate genotoxicological end points produced by 2-nitropropane (Figure 3).[57] It was concluded that the toxic effects of 2-nitropropane are not mediated by reductive metabolites nor reductive metabolic processes. It should be noted, however, that acetone oxime (**15**) is tumorogenic in rats[58] and that acetone oxime was later proposed[59] as an

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intermediate in the activation of 2-nitropropane to a carcinogen. In contrast to Haas-Jobelius et al.,[57] other investigators[60] did find that acetone oxime (**15**) caused modification to DNA and RNA bases in rats.

Several studies have shown that 2-nitropropane (**12**) is mutagenic in the Ames mutagenicity assay.[61−65] In 1987, it was reported that 2-nitropropane nitronate, the tautomeric form (Scheme 1) of 2-nitropropane, was more mutagenic in the Ames mutagenicity assay than neutral 2-nitroproane[63] and that mutagenicity of propane-2-nitronate was proportional to incremental increases of the incubation buffer pH.[66] Because the pK_a of 2-nitropropane is 7.6, higher pH values favor the formation of propane-2-nitronate. In addition, the mutagenicity of 2-deutero-2-nitropropane was lower than 2-nitropropane, a fact that also associated the formation of 2-nitropropanate with toxicity end points. These data were consistent with studies indicating that nitronates of secondary nitroalkanes were consistently more mutagenic than their respective neutral nitroalkanes[65] and that primary neutral and nitronate alkanes were not mutagenic or were marginally mutagenic.[65] The mutagenicity of propane-2-nitronate in *Salmonella* strain TA102,[63,65] which was developed to detect oxidative damage to DNA,[67] suggested that free radical mechanisms of 2-nitropropane activation might cause toxicity.[63,65] Mutagenicity, however, was also correlated to the slow rate of protonation of the nitronate anion of secondary nitroalkanes[68] and inversely related to the very rapid rate of protonation of the primary nitroalkane and nitrocarbinol nitronates.[65] In rat hepatoma cells, propane-2-nitronate was denitrified more rapidly than equal amounts of 2-nitropropane, and enhancements of DNA-repair mechanisms[69] were greater in cells treated with 2-nitropropanate. Collectively, these data suggested that the isomeric form of a secondary nitroalkane present intracellularly was critical to toxicity but that the ultimate mechanism of the toxicity might be through one of several mechanisms.[56]

Of primary interest to regulatory and industrial toxicologists are the genotoxic and carcinogenic end points that 2-nitropropane has consistently induced in test animals. In rats, oxidative damage to DNA and RNA occurs after administration of 2-nitropropane, but not after treatment with primary nitroalkanes. For example, levels of 8-oxodeoxyguanosine (**18**; Figure 4) (DNA modification) and 8-oxoguanosine (**19**) (RNA modification) were increased 3.6- and 11-fold in treated rats compared to controls,[70] as was the appearance of two unidentified oxidized nucleoside products in each of RNA (RX1, RX2) and DNA hydrosylates (DX1, DX2). The presence of the oxidized RNA and DNA products correlated with patterns of nitroalkane genotoxicity. For example, secondary nitroalkanes, but not primary or tertiary nitroalkanes,[68] increased RX1, RX2, DX1, and DX2 production in rat livers. Female rats, which are less susceptible to 2-nitropropane carcinogenicity than male rats, had significantly less RNA and DNA modification than male rats.[71] In a similar manner, DNA and RNA modifications were produced in livers (the target tissue for 2-nitropropane), but were minimal in nontarget organs such as the kidney.[71] In addition, levels of the base modifications in rabbits were very low,[72] consistent with the refractory nature of rabbits to 2-nitropropane-induced carcinogenicity.[73]

Additional nucleoside modifications produced by exposure to 2-nitropropane were identified as 8-aminoguanosine (**20**) and 8-aminoguanine (**21**) by Sodum et al.[59] (Figure 4). A third, and somewhat novel, oxidation product, 8-aminodeoxyguanosine (**22**), was also characterized in DNA from 2-nitropropane-treated rats. Sodum's report that amination occurred at the 8-position of guanine under physiologic, albeit carcinogenic, conditions was the first description of such a base modification. Amination of guanine via metabolism of 2-nitropropane was proposed[59] to occur through the formation of the highly reactive nitrenium ion (NH_2^+; **23**) formed from the nitro group of 2-nitropropane (**12**) (Scheme 7). Sodum et al.[59] further proposed a mechanism through which both both 8-oxoguanine and 8-aminoguanine could be produced via a common aziridine intermediate, formed from the reaction of guanine and the nitrenium ion (scheme not shown).

Sodum's hypothesis was that propane-2-nitronate (**12a**) could be activated through nitrogen hydroxyl conjugation with

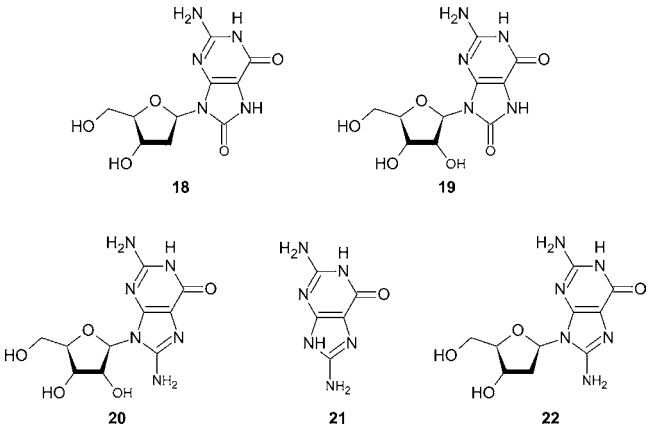
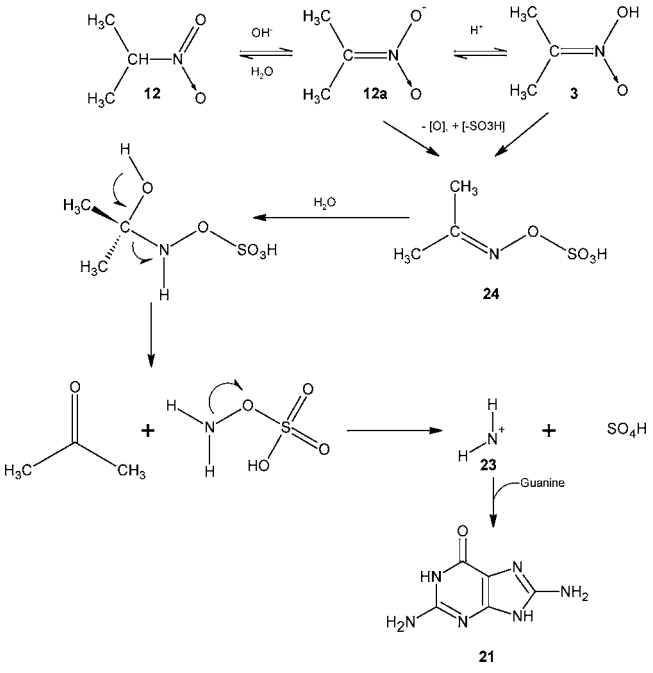


Figure 4. Structures of 8-oxodeoxyguanosine (**18**), 8-oxoguanosine (**19**), 8-aminoguanosine (**20**), 8-aminoguanine (**21**), and 8-amino-deoxyguanosine (**22**).

Scheme 7. Proposed Formation of 8-Aminoguanine (21) through Formation of an Acetone Oxime Sulfate Conjugate (Acetyl Conjugate Not Shown) (Adapted from Reference 59)



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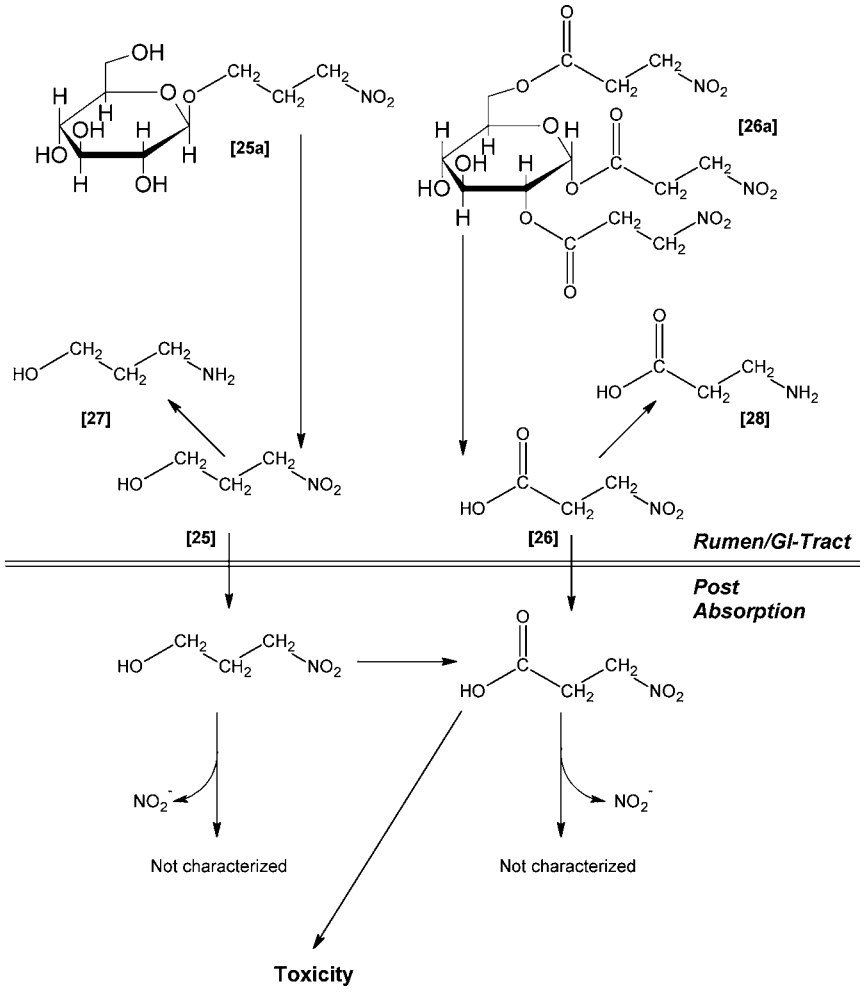
Scheme 8. Ruminal Metabolism of 3-Nitropropanol and 3-Nitropropionic Acid Glycones



either sulfate or acetate, presumably through the action of sulfotransferases or acetyltransferases on propane-2-nitronate or acetone oxime (**15**).[59] Although chemical evidence that hydroxylamine-*O*-sulfonic acid (**24**) reacts with DNA and RNA to produce 8-aminoguanine was provided by Sodum et al.,[59] no evidence to support the initial events (loss of oxygen and conjugation) of the hypothesis was presented. In addition, no evidence of the physiologic conjugation of propane-2-nitronate (**12a**) or acetone oxime (**15**) was provided. Subsequent investigation did establish circumstantial evidence for the involvement of sulfotransferases in 2-nitropropane activation. For example, pretreatment of rats with ayrl sulfotransferase inhibitors significantly reduced the formation of nucleoside modifications in livers of rats treated with 2-nitropropane, whereas inhibitors of alcohol sulfotransferase had no effect.[74] In addition, partially purified aryl sulfotaransferase mediated the formation of nucleoside base modifications in the presence of 2-nitropropane and propane-2-nitronate, but not in the absence of sulfotransferase cofactors or with denatured enzyme.[74] Consistent with in vivo toxicity studies, aryl sulfotransferases were able to increase base modifications with a variety secondary nitroalkanes and alkane nitronates, but not with primary alkanes or primary nitroalkonates.[75]

Andrae et al.[76] unambiguously established the role of sulfotransferases in 2-nitroproane activation using V79-derived cells transected with specific rat sulfotransferase isozymes. Hydroxysteroid sulfotransferases were devoid of the ability to activate propane-2-nitronate, whereas sulfotransferases 1A1 and 1C1 were highly efficient nitronate activators. In addition, none of the sulfotransferases activated acetone oxime (**15**), a result that established the sequence of sulfotransferase activation. Sulfotransferases activate the nitronate anion directly, and acetone oxime is not an intermediate (i.e., sulfation occurs before the oxygen loss shown in Scheme 7). Essentially identical results were obtained with human sulfotransferases transfected into V79 cells.[77] Loss of water from acetone oxime sulfonate (**24**) as well as the subsequent steps leading to the formation of NH_2^+ (**23**) occur nonenzymatically in a pH-dependent manner as demonstrated by Sodum and Fiala[78] (Scheme 7). The ability of human sulfotransferases to activate 2-nitropropane strongly supports the supposition that 2-nitropropane would be a carcinogen in humans.

ADME of Longer Chain Secondary Alkanes. In vivo absorption, disposition, metabolism, and excretion studies for secondary nitroalkanes of greater than three carbons have not been published.

ADME of 3-Nitropropanol and 3-Nitropropionic Acid. Two naturally occurring nitrotoxins, 3-nitropropanol (3-nitropropan-1-ol) (**25**) and 3-nitropropionic acid (**26**), will be discussed together because of their similar modes of action. Of relevance to a discussion on the ADME of each compound is the fact that 3-nitropropionic acid is toxic to both nonruminants and ruminants, but it is much less toxic to ruminants than nonruminants after oral administration. In contrast, 3-nitro-

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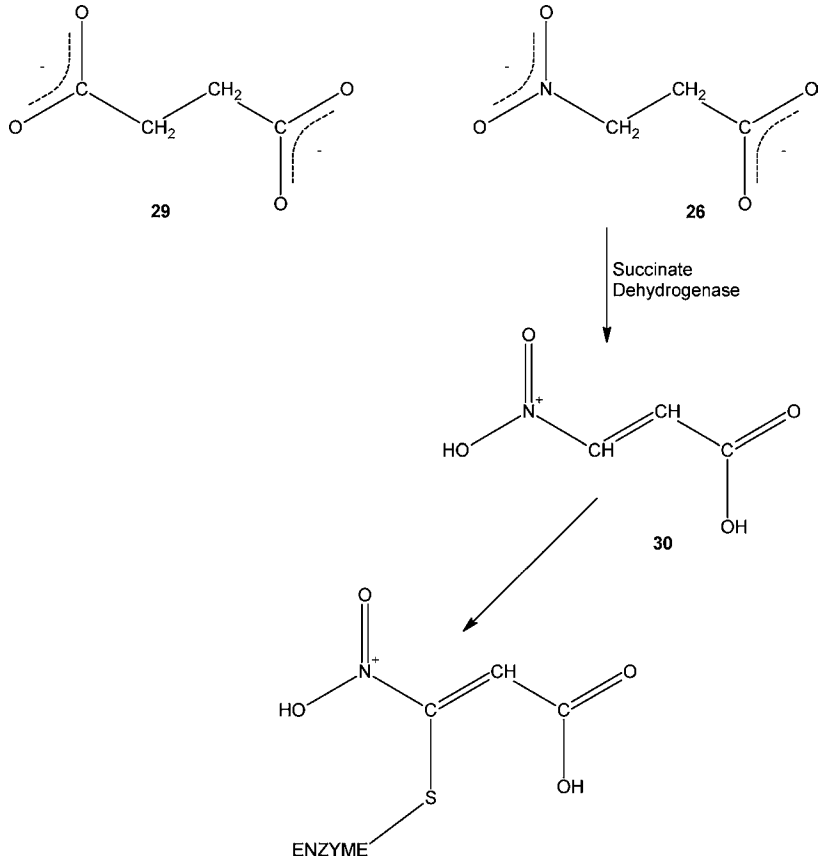
Scheme 9. Proposed Mechanism for 3-Nitropropanoic Acid Toxicity



propanol toxicity is roughly equivalent in ruminants and nonruminants.[17] Both toxins are of economic importance to the livestock industry. Although considerable efforts have been extended in understanding the mechanisms of these toxins, classical radiolabeled ADME studies have not been conducted for either compound.

Scheme 8 summarizes the biotransformation of 3-nitropropanol and 3-nitropropionic acid in ruminants and nonruminants. In ruminants, glycosides of both 3-nitropropanol (miserotoxin (**25a**))[79] and 3-nitropropionic acid (**26a**)[80] are rapidly hydrolyzed to aglycones by bacterial glycosidases and esterases. Ruminal or gastrointestinal reduction of aglycone nitro groups of the carbinol and acid produce their respective amines, 3-aminopropan-1-ol (**27**) and 3-aminopropionic acid (β-alanine) (**28**).[81] Rates of ruminal metabolism of nitropropanol may be manipulated by dietary source,[82] addition of nitroethane, and adaptation of ruminal bacteria to timber milkvetch (a source of miserotoxin).[83] Thus, ruminal bacterial populations can be adapted to the presence of nitroaliphatic alcohols and (or) acids. Of significance to toxicity in ruminant animals, the rate of ruminal 3-nitropropionic acid metabolism is much greater than the rate of 3-nitropropanol transformation.[84,85] Slower relative rates of ruminal 3-nitropropanol metabolism allow a greater degree of 3-nitropropanol absorption than 3-nitropropionic acid. Definitive radiolabeled metabolism studies have not been conducted to determine the absolute fate of either 3-nitropropionic acid or 3-nitropropanol in ruminal systems. However, Anderson et al.[81] have proposed that β-alinine (**28**) is produced by the ruminal reduction of 3-nitropropionic acid and that subsequent intermediary metabolism of β-alanine results in carbon incorporation into natural products. The ruminal fate of 3-aminopropanol (**25**) is unknown. Although it is established that the ruminal denitrification of the acid and the alcohol can occur,[85] the total extent of denitrification and the pathways through which the carbon backbone is metabolized are unknown. Ruminal[86] and soil[87] bacteria have been isolated that can use 3-nitropropanol and (or) 3-nitropropionic acid as a source of carbon, nitrogen, and energy under anaerobic and aerobic conditions, respectfully.

After ruminal hydrolysis of the nitrocarbinol and nitroacid glycosides, free nitrocompounds are rapidly absorbed from the rumen.[88,89] In cattle, the ruminal half-life of 3-nitropropanol was just over an hour and the appearance of 3-nitropropionic acid in serum peaked within 0.5−1 h of dosing.[88] Although the rumen is the major site of absorption, Pass et al.[89] demonstrated that essentially the entire length of the ruminant gastrointestinal tract is capable of accommodating rapid 3-nitropropanol absorption. Once absorbed, 3-nitropropanol has a very short plasma half-life of $\leq$10 min[89,90] because it is very rapidly oxidized to 3-nitropropionic acid, presumably by aldehyde dehydrogenases.[36,91] In contrast, 3-nitropropionic acid is metabolized at a much slower rate in cattle than is 3-nitropropanol.[92] In both cattle and rats, the only reported biotransformation product reported has been nitrite, the product of denitrificaton.[42,89]

The importance of whole-animal metabolism in initiating the toxic events of miserotoxin poisoning has been demonstrated in a series of rodent studies. First, intact miserotoxin itself (3-nitro-1-propyl-β-D-glucoside (**25a**)), although well absorbed, exhibited very low toxicity, having an oral LD_{50} of >2500 mg/kg bw relative to the aglycone, which had an LD_{50} of 77 mg/kg

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Scheme 10. Mechanism of 2-Nitropropanol Toxicity Proposed by Alston et al.[101]

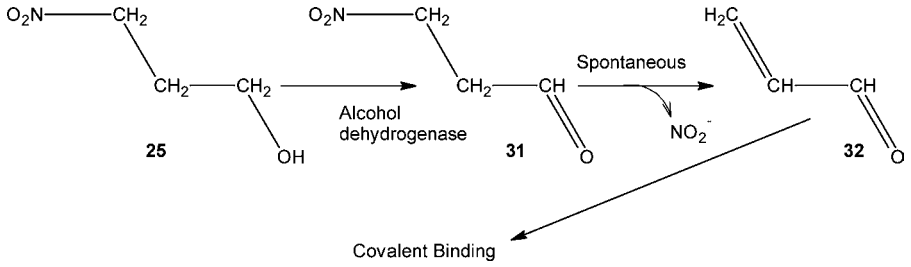

Table 6. Disposition of Radioactivity in Rats and Beagles Administered a Single Oral Dose (1 mg/kg bw) of [2-^{14}C]-Bronopol (Adapted from Reference 104)

			recovery of radioactivity (% of dose)		
species	reps	fraction	0−24 h	0−48 h	0−120 h
rat	6	urine	80.9 ± 2.0	82.7 ± 2.1	83.3 ± 2.1
		feces			5.8 ± 0.8
		expired air	6.3 ± 0.6	8.0 ± 0.9	
		carcass			1.6 ± 0.2
		cage wash			0.9 ± 0.3
		recovery			99.6
rat	2	urine		72.0 ± 12.0	
		bile	4.0 ± 3.0	4.6 ± 3.7	
		recovery		76.6 ± 8.3	
dog	2	urine	77.1 ± 10.3	79.6 ± 10.3	81.2 ± 10.1
		feces		2.6 ± 0.6	3.1 ± 0.4
		cage wash			0.6 ± 0.4
		recovery			84.9 ± 10.1

bw.[93] Whereas 3-nitropropanol was much more toxic than its glucose conjugate, toxicity was conferred only after oxidation of 3-nitropropanol to 3-nitropropionic acid. Thus, prior administration of alcohol dehydrogenase inhibitors prevented 3-nitropropanol toxicity,[91] and sequential administration of 3-nitropropanol followed by alcohol dehydrogenase inhibitors did not ameliorate toxicity.[89] Consistent with the hypothesis that 3-nitropropionic acid mediates 2-nitropropanol toxicity, the LD_{50} values of 3-nitropropanol and 3-nitropropanoic acid were essentially identical after parenteral administration.[91]

Toxic end points caused by 3-nitropropionic acid in affected animals are driven by separate biochemical mechanisms. Systemic denitrification of 3-nitropropanoic acid, and possibly limited amounts of 3-nitropropanol, yields nitrite, a chemical oxidant that catalyzes the transformation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron on hemoglobin to form methemoglobin.[42,94] Methemoglobin is incapable of transporting oxygen,[95] and blood methemoglobinemia burdens of 80% in animals[96] and 50% in humans[97] may cause cyanosis, resulting in asyphyxia. In 1961 Matsumoto et al.[42] administered 28 mg/kg bw 3-nitropropionic acid to rats via intraperitoneal or intravenous injection and measured maximal methemoglobin levels of approximately 30% within 2 h of dosing. Rats dosed intraperitoneally with 3-nitropropanoic acid showed severe signs of poisoning, and only 1 of 15 rats survived the same dose when administered intravenously. In the same study, equivalent doses of nitrite caused >50% methemoglobinemia, but with few observable negative outcomes. Matsumoto et al.[46] concluded that severe morbidity and subsequent mortality of 3-nitropropionic acid dosed rats was not occurring through methemoglobin formation. Similar conclusions were surmised after studies investigating the toxicity of *Astragalus* species in avian and ruminant species in the United States.[98] It is generally recognized that the methemoglobinemia produced by 3-nitropropionic acid is nonlethal.[17]

Lethality is, however, commonly associated with the consumption of *Astragulus* and *Coronilla* species. Administration of miserotoxin and 3-nitropropionyl glycones and the experimental administration of 3-nitropropanol and 3-nitropropionic acid[17,18] to test animals are also lethal. As early as 1964, Hylin and Matsumoto[99] proposed that 3-nitropropionic acid mediates severe toxicity through the competitive inhibition of succinate dehydrogenase. But it was not until 1977 that Alston et al.[35] showed that 3-nitropropionic acid is a suicide inhibitor of succinate dehydrogenase and proposed this to be the mechanism through which 3-nitropropionic acid causes severe toxicity. As shown in Scheme 9, 3-nitropropionic acid (**26**) is a structural analogue of succinate (**29**); 3-nitropropionic acid is hypothesized to be metabolized by succinate dehydrogenase to 3-nitroacrylic acid (**30**). Enzyme inactivation occurs through irreversible binding of 3-nitroacrylic acid to the active site of succinate dehydrogenase. Alston et al.[35] proposed that inactivation would occur due to covalent binding onto the flavin cofactor of the enzyme, whereas Coles et al.[100] proposed covalent binding to an active site thiol.

Alston et al.[101] further hypothesized a mechanism through which 3-nitropropanol could directly affect toxicity through metabolism of the nitroalcohol by alcohol dehydrogenase. Specifically, alcohol dehydrogenase oxidation of 3-nitropropanol (**25**) to 3-nitropropionaldehyde (**31**) followed by

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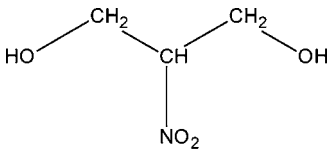
its nonenzymatic decay to nitrite and acrolein (**32**) (Scheme 10) was proposed. Acrolein is an unstable electrophile capable of covalently binding to nucleophilic regions of proteins and other tissue macromolecules. As such, it has systemic and neurological toxicity,[102] but it is not characterized as a strong mutagen or carcinogen.[103] To date, the hypothesis of direct activation of 3-nitropropanol has not gained much attention, perhaps because (1) 3-nitropropanol is very rapidly oxidized to 3-nitropropionic acid during systemic circulation; (2) the appearance of nitrite in the blood of 3-nitropropanol affected animals is correlated with the appearance of 3-nitropropionic acid, and not 3-nitropropanol; and (3) the clinical end points of 3-nitropropanol toxicity and 3-nitropropionic acid toxicity are similar.

ADME of Bronopol (2-Bromo-2-nitro-1,3-diol; 1). Moore et al.[104] orally administered 1 mg/kg bw of [2-^{14}C]Bronopol to both rats and beagle dogs and followed the disposition of radioactivity for 5 days. Expired radioactivity was measured in rats, but not in dogs. In addition, a set of rats was also bile duct cannulated, and radioactivity in urine, feces, and bile was measured over a 48 h period. Data from the study are shown in Table 6. In both rats and dogs, the major route of elimination was urine, with >90% of the total urinary excretion occurring during the 24 h immediately following dosing. Unlike the metabolism of nitroalkanes, loss of radioactivity through the lung as $^{14}CO_2$ was a minor route of Bronopol elimination. Similar in magnitude to respired radioactivity, only about 2−7% of the dosed radiocarbon was eliminated in bile of rats. Distributions of radioactivity in rats and dogs were similar. There was no evidence that radioactivity preferentially accumulated in any specific tissue or organ. In rats killed 1.5 and 6 h after dosing, levels of radioactivity in kidneys were greater than other tissues, but this was likely due to the active renal elimination that occurred during the initial 24 h after dosing. Parent Bronopol was not excreted in the urine of rats or dogs; rather, a single major metabolite, 2-nitropropane-1,3-diol (**33**; Figure 5), represented approximately 43% of the urinary



33

Figure 5. Major metabolite of Bronopol, 2-nitropropane-1,3-diol (**33**).

radioactivity. One other major metabolite (~10.5% of urinary radioactivity) and three minor metabolites (~3−4% of urinary radioactivity each) were excreted in urine, but were not identified. The formation of 2-nitropropane-1,3-diol (**33**) from parent Bronopol occurred spontaneously in vitro in the presence of thiols. In dog or rat serum, Bronopol was completely debrominated to 2-nitropropane-1,3-diol (**33**) within 5 min. Thus, the major excretory product of Bronopol is formed spontaneously through nonenzymatic mechanisms.[104]

Because a major use of Bronopol is as an antimicrobial of dermal cosmetics, Moore et al.[105] studied its fate after dermal administration to rats and rabbits. Both species were dosed with 1 mg/kg bw Bronopol, labeled with ^{14}C in the 2-position, and urine, feces, and respiratory gases were collected for a period of up to 48 h. Sites of dosing were covered with an occlusive dressing composed of polyethylene. Dermal absorption of Bronopol was dependent upon the excipient with about 20% of the radioactivity absorbed when applied in neat acetone, 7% absorption when applied in acetone/water (9:1), and 10% absorption when applied in 100% water. Regardless of the excipient, urine was always the major route of excretion of absorbed radioactivity. Most of the applied dose (80−90%) remained associated with the site of application. Metabolites excreted in urine were identical to metabolites excreted in urine of rats after oral administration.

Buttar and Downe[106] compared the disposition of [2-^{14}C]-Bronopol (10 mg/kg bw) after dermal and intravenous administration to rats. Bronopol was applied to the skin of rats as an acetone solution. In contrast to Moore et al.,[105] these investigators covered the dermal dosing sites with a non-occlusive dressing composed of a plastic material. The disposition of radioactivity in test animals is shown in Table 7. The authors calculated total absorption to be 40% after

Table 7. Cumulative Disposition (24 h) of Radioactivity in Rats after Dermal or Intravenous Administration of a Single [2-^{14}C]Bronopol Dose (10 mg/kg bw) to Rats[106]

	recovery of radioactivity (% of dose)	
fraction	dermal[a]	intravenous[b]
urine	15.1 ± 1.6	74.0 ± 1.9
feces	0.9 ± 0.2	1.3 ± 0.1
expired air	2.1 ± 0.2	9.0 ± 0.5
cage wash	0.6 ± 0.2	0.5 ± 0.3
total	18.7 ± 1.7	84.8 ± 1.4
skin (application site)	59.5 ± 3.4	NA[c]

[a]Mean ± standard error of the mean of seven animals. [b]Mean ± standard error of the mean of 6 animals. [c]Not applicable.

dermal application; however, this value was calculated by difference from skin radioactivity and total applied radioactivity. Only 19% of the applied dose was measured in absorbed fractions. The measured value is highly consistent with the 20% absorption measured after dermal application of an acetone solution of Bronopol to rats.[105] Distribution of radioactivity in rats dosed intravenously was comparable to the disposition of radioactivity after oral administration.[104] Parent Bronopol was not present in the urine of dermally or intravenously dosed rats, and urinary 2-nitropropane-1,3-diol (**33**) represented 53% of the dosed radioactivity in the intravenously dosed animals. Two other metabolites were not identified.

An additional metabolism and disposition study with [2-^{14}C]Bronopol was published in 1987 by Kujawa et al.,[107] who orally dosed rats with a larger dose (25 mg/kg bw) than did Moore et al.[104] and investigated the fate of Bronopol after repeated oral application in rats. In addition, a number of end points not previously investigated were measured, including sex differences and plasma kinetics. Figure 6 shows the absorption and depletion of total radioactive residues and of 2-nitropropane-1,3-diol (**33**) in male and female rats. By 24 h, 2-nitropropane-1,3-diol was not detectable in blood; unidentified metabolites that comprised the total radioactive residue fraction were clearly present at 24 h and were still detectable at 72 h (data not shown). No appreciable sex differences were noted in the disposition of Bronopol, but it was noted that after repeated (5×) doses of nonlabeled Bronopol, the amount of radiocarbon retained by tissues after a single dose of radiolabeled Bronopol increased approximately 3-fold.

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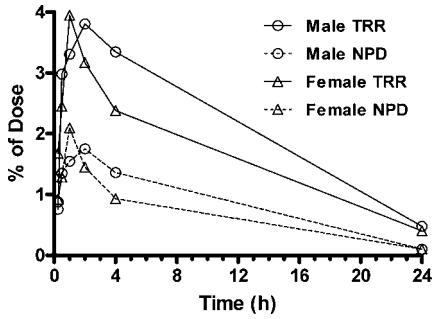


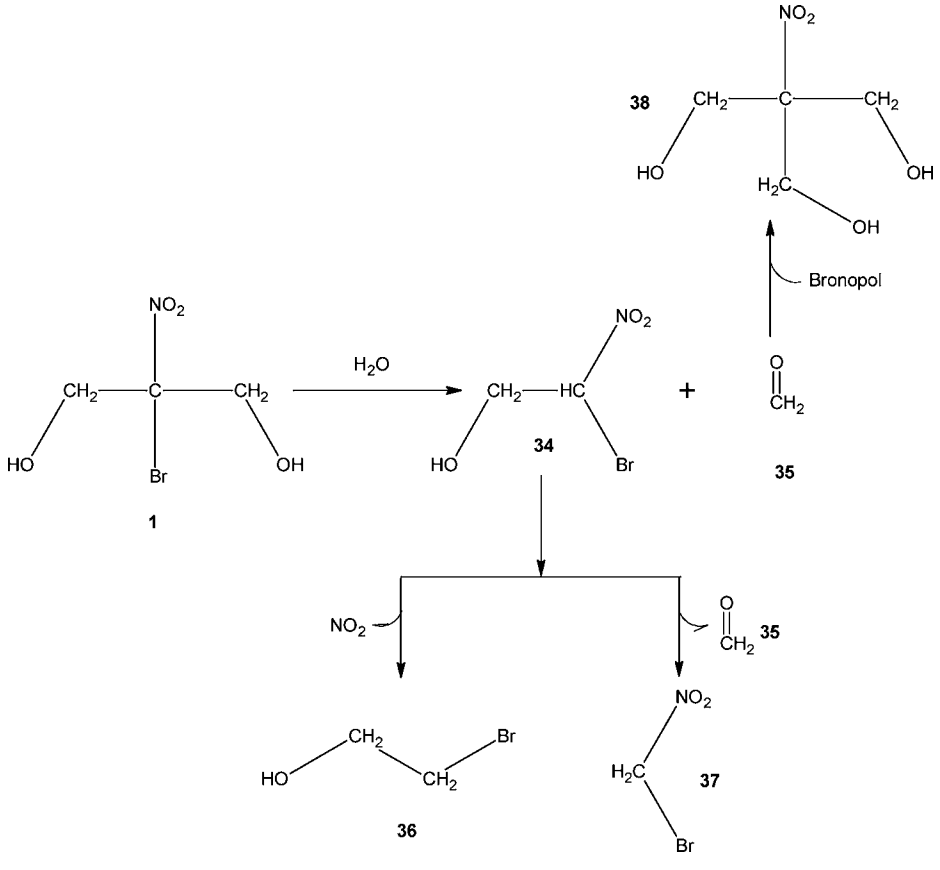
Figure 6. Kinetics of total radioactive residues (TRR) and of 2-nitropropane-1,3-diol (NPD) (**33**) in male and female rats after oral administration of a 25 mg/kg bw dose (adapted from ref 107).

The U.S. Environmental Protection Agency (U.S. EPA) summarized a number of unpublished metabolism and disposition studies on Bronopol in rats.[32] Studies summarized in the EPA review, although conducted with some variables that differed from published studies, came to basically the same conclusions as published studies. Namely, Bronopol is rapidly absorbed and is rapidly debrominated to form 2-nitropropane-1,3-diol, which is rapidly excreted in urine as the major metabolite. The EPA concluded that fecal and respiratory eliminations of Bronopol metabolites represent minor routes of elimination. Other than 2-nitropropane-1,3-diol, the chemical identity of minor Bronopol metabolites remains unknown.

Bronopol is generally regarded as having low systemic toxicity,[32,108] but its use in consumer products, especially cosmetics, has shown that repeated application can lead to contact dermatitis[109] in some individuals. Although biotransformation of Bronopol is not thought to play a role in sensitization, Bronopol degradation pathways are thought to contribute to sensitization issues. When stored in the dark at room temperature, crystalline Bronopol is essentially stable for years, but in aqueous solution degradation occurs in a temperature- and pH-dependent manner.[108] At 20 °C, the half-lives of aqueous Bronopol (0.2%) at pH 4, 6, and 8 were >5 years, 1.5 years, and 2 months, respectively. The initial events in bromopol decomposition in aqueous solution were described as a retroaldol reaction with the release of 2-bromo-2-nitroethanol (**34**) and formaldehyde (**35**) (Scheme 11). 2-Bromo-2-nitroethanol (**34**) itself is reactive and may decompose to 2-bromoethanol (**36**) or bromonitromethane (**37**).[108,110] Formaldehyde (**35**) formed from the initial decomposition of Bronopol or from the decomposition of 2-bromo-2-nitroethanol (**35**) can react with Bronopol to form tris(hydroxymethyl) nitromethane (**38**).

From a toxicological perspective, the release of formaldehyde during the degradation of Bronopol is of concern because formaldehyde is a common contact allergen.[111] At least a portion of the contact sensitivity to Bronopol is attributable to formaldehyde (see discussion of Kireche et al.[110]), but a large number of individuals sensitive to Bronopol are not formaldehyde sensitive. Kireche et al.[110,112] have proposed that intermediates other than formaldehyde that are formed during Bronopol degradation are capable of haptenization of tissue proteins. They demonstrated that although Bronopol degrades very slowly at physiologic pH, the degradation products bromoethanol (**34**) and bromonitromethane (**37**) were capable

Scheme 11. Aqueous Degradation of Bronopol (Adapted from References 108 and 110)



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of binding to free amino acids, especially lysine and cysteine. They hypothesized that the degradation products would also bind to lysine and cysteine residues in proteins. Although these compounds were amino acid reactive, only bromonitromethane (**37**) was toxicologically active in cell models.[112] Both bromonitromethane (**37**) and bromonitroethanol (**34**) are present in formulated retail shampoos, cosmetics, and veterinary products.[113]

CONCLUSIONS

Short-chain nitroalkanes, nitroalcohols, and nitroacids and brominated nitroalcohols have been reviewed in the context of their most important toxicolgocial properties and mechanisms of toxicity as related to metabolism or degradation pathways. With the exception of the propensity for denitrificaion with the subsequent oxidation of hemoglobin by nitrite to produce acute methemoglobin, individual nitrocompounds share few toxicological end points. For example, few data support the genotoxic potential of primary or tertiary nitroalkanes, nitroalcohols, or nitroacids. Secondary alkanes appear to be genotoxic through specific enzyme-mediated pathways, which are not shared with secondary nitroalcohols or nitroacids. A seemingly unique primary nitroacid, namely, 3-nitropropanoic acid, is highly toxic through its specific inhibition of succinate dehydrogenase. Other nitroacids have not been shown to inhibit succinate dehydrogenase. The corresponding nitroalcohol, 3-nitropropan-1-ol, produced toxicity similar to that of 3-nitropropanoic acid, but only after it is metabolized to 3-nitropropionic acid by alcohol dehydrogenases. Other nitrocompounds appear to be well tolerated toxciologically and have enjoyed diverse uses in industrial, cosmetic, and agricultural applications.

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Notes

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