U.S. Army Public Health Center

Wildlife Toxicity Assessment for Mono-Nitrophenols

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Toxicology Directorate, Health Effects Division

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This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of the mono-nitrophenols on wildlife. Evaluating the toxicity of nitrophenols will contribute to the derivation of toxicity reference values (TRVs) for use in assessing potential health effects for wildlife near contaminated sites. The U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, Standard Practice for Wildlife Toxicity Reference Values (USACHPPM, 2000) documents the protocol for the performance of this assessment. Any derived TRVs in this document are intended for use by toxicologists and risk assessors to develop environmental health risk management strategies.

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Department of the Army
U.S. Army Public Health Center

Wildlife Toxicity Assessment for Mono-Nitrophenols

CAS No. 25154-55-6 April 2020

1. INTRODUCTION

This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of the mono-nitrophenols on wildlife. Evaluating the toxicity of nitrophenols will contribute to the derivation of toxicity reference values (TRVs) for use in assessing potential health effects for wildlife near contaminated sites. The U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM, 2000) documents the protocol for the performance of this assessment. Any derived TRVs in this document are intended for use by toxicologists and risk assessors to develop environmental health risk management strategies. The TRVs developed throughout this document represent the likelihood of toxic effects in individual organisms that have population-level effects, but do not extend to demographic rates for specific populations. Relevant studies used for TRV derivation have several key features: (1) any toxic effects identified are linked to potential population-level effects; (2) exposure duration is clearly identified; (3) effect levels are reported as no-observable-adverse-effect-level (NOAEL), lowest-observable-adverse-effect-level (LOAEL), effect dose; (4) exposure pathway is relevant to wildlife or exposure in the field; (5) validity and quality of the study are appropriate for inclusion in use for TRV derivation.

Three isomers of mono-nitrophenol exist: *ortho*- or 2-nitrophenol*, meta*- or 3-nitrophenol, and *para*- or 4-nitrophenol. Nitrophenols may be released into the environment during their use as organic intermediates and indicators (von Stackleberg et al. 2006). 2-Nitrophenol is used mainly as an intermediate for the production of dyestuffs, pigments, rubber chemicals, and fungicides. Small amounts are used as an acid-base indicator and as a reagent for glucose (ATSDR, 1992; von Stackleberg et al. 2006). The major applications of 4-nitrophenol are in the context of an important intermediate in the synthesis of a variety of organic compounds. These include the manufacture of pesticides, fungicides, paints, dyes, leather preservative, and drugs, which can easily become anthropogenic pollutants.

Both 2-nitrophenol and 4-nitrophenol are likely to threaten the environment and public health (Bielska and Szymanowski, 2004). The use pattern of 4-nitrophenol is summarized as follows: production of N-acetyl-4-aminophenol – approximately 55%; exports – approximately 35%; and miscellaneous uses to include leather tanning, insecticides (methyl and ethyl parathion), dye-stuffs and oxydianiline manufacture – approximately 10%. Low levels of 4-nitrophenol are used as a laboratory reagent (e.g., phosphatase and carboxyesterase determinations) and as a fungicide in military footwear. Presently, 4-nitrophenol is registered with the U.S. Environmental Protection Agency (U.S. EPA) for fungicidal use, and was first registered for this use in 1963 (ATSDR, 1992; U.S. EPA, 1998; von Stackleberg et al. 2006). Registration for 4-nitrophenol as

a fungicide was granted in 1980 when incorporated into leather for military use, and at a concentration not to exceed 0.7% on the basis of dry finished leather weight. In 1983, an amendment to this registration permitted use of 4-nitrophenol into cork insulation for military use (U.S. EPA, 1998). In addition, both the 2- and 4- isomers may be released in vehicular exhaust from gasoline and diesel engines (von Stackleberg et al. 2006).

2. TOXICITY PROFILE

2.1 Literature Review

Electronic searches of relevant biomedical, toxicological, and ecological databases (e.g., BIOSIS, Defense Technical Information Center (DTIC®) On-Line Multisearch, and TOXNET®) occurred on October 9–November 21, 2013, and again in November 29–30, 2017 and July 1–3, 2019 during the revision of this draft, to identify primary reports of studies and reviews on the toxicology of nitrophenols. Separate searches were conducted for general toxicology as well as specific searches for birds, reptiles, amphibians, and wildlife. Each database was searched using keywords that included nitrophenol, 2-nitrophenol, 3-nitrophenol, or 4-nitrophenol or their Chemical Abstracts Service Registry Numbers (CAS RNs) (25154-55-6, 88-75-5, 554-84-7, or 100-02-7, respectively) plus toxicity, eco-toxicology, wildlife, avian, bird, frog, amphibian, or reptile. Appendix B documents the details and results of the search strategy. The titles of articles identified in each search were reviewed for relevance. Potentially relevant articles focused on toxic effects on terrestrial vertebrates or environmental fate of nitrophenols. All likely relevant articles were acquired as electronic files or by visiting the Welch library of the Johns Hopkins University School of Medicine. Review articles provided additional material not identified during the initial literature searches and screening. Studies were classified based on organism, route, and duration of exposure. For the purpose of this WTA: acute studies are defined as single or repeat exposures for less than 14 days or 10% of the life span of the organism; subchronic studies are defined as repeat exposures that are greater than 14 days and less than 10% of the life span of the organism; and chronic studies are defined as those equal to or greater than 10% of the life span of the organism. If exposure occurs during a sensitive life stage (e.g., gestation), then a classification as chronic is appropriate for related endpoints (e.g., early development, litter size) (USACHPPM 2000).

2.2 Environmental Fate and Transport

Release of mono-nitrophenols to the environment, and particularly to waste water columns, occurs largely during their production and use as rubber chemicals, chemical indicators, and intermediates for various dyes, paint colorings and stains, fungicides, and pharmaceuticals. Photooxidation of aromatic hydrocarbons including benzene and toluene with nitric oxides in the ambient air liberates both 2- and 4-nitrophenol, and both isomers have been detected in gasoline and diesel engine vehicular exhaust emissions (HSDB, 2013a–d). The isomer 4-nitrophenol is a degradation product of parathion and is present as an impurity in thiophos – a parathion formulated compound. Further, 4-nitrophenol will be formed in the ambient air following reaction of nitrate free radicals with phenol.

On release to the ambient atmosphere, nitrophenols are present in both the vapor and particulate phases at vapor pressures of 5 x 10^{-4} to 0.100 mm Hg at 25ºC (HSDB 2013a–d). Particulate-phase nitrophenols may also be removed from the air by wet and dry deposition. Vapor-phase nitrophenols are also removed from the ambient air by dissolution into cloud water vapors and subsequent precipitation. Additionally, vapor-phase nitrophenols are removed from the ambient atmosphere by dissolution into cloud vapors and precipitation.

Isomers of nitrophenols are manufactured organic compounds, which do not occur naturally in the environment (HSDB 2013a). Nitrophenol isomers are differentially water-soluble with log KOW values, and all are solids at room temperature (Table 1). Nitrophenol isomers are moderately acidic in aqueous media due to their dissociation properties. Both 3- and 4-nitrophenol have low vapor pressures, although 2-nitrophenol has a vapor pressure that is somewhat higher than the meta- and para-isomers. The environmental consequences of these values are described in somewhat more detail in the sections below.

Available monitoring data for 2-nitrophenol informs that it exists in both the vapor-phase and particulate phase in the atmosphere. On release to the soil, 2-nitrophenol is predicted to display high to moderate mobility given its log K_{OC} values of 1.1–2.4. The K_{OC} values also suggest that on release to water columns, 2-nitrophenol would have minimal to moderate adsorption to solids in suspension and to the sediment. Moreover, the Henry's Law constant of 1.63 x 10^{-5} atmospheres per cubic meter per mole (atm-m^3/mole) (25ºC; Table 1) indicates that volatilization of 2-nitrophenol from the surfaces of moist soil could be an important environmental fate pathway. It is not expected that 2-nitrophenol will undergo hydrolysis in the environment due to a lack of available functional groups that can be hydrolyzed (HSDB 2013b). The measured K_{OC} values also suggest that 2-nitrophenol might have moderate to very high mobility in soil although 2-nitrophenol is not expected to volatize from dry soil surfaces since its vapor pressure is 0.100 millimeters of mercury (mm Hg) at 25ºC. Others had previously reported that 2-nitrophenol completely biodegrades in an aqueous soil media within 7–14 days (Haller 1978). By contrast, 2-nitrophenol was shown to require in excess of 64 days to biodegrade when present in the soil (Alexander and Lustigman 1966).

Available data for 3-nitrophenol informs that it can be released to the environment through various waste water columns via its production and use as an intermediate (HSDB 2013c). On release to the air, an estimated vapor pressure of 1.5 x 10^{-4} mm Hg at 25ºC suggests that it will exist exclusively as a vapor in the ambient air. At temperatures less than 20ºC, 3-nitrophenol will be present in both the vapor and particulate phases. In the vapor phase, 3-nitrophenol will break down in the atmosphere following its reaction with photochemically synthesized hydroxyl radicals. The measured K_{OC} values suggest that 3-nitrophenol might have moderate to high mobility in the soil. The K_{OC} values of 3-nitrophenol also suggest that if released into the water, it may have little to moderate adsorption to suspended solids and sediment in the water column.

Volatilization from water surfaces is not expected to be an important environmental fate process for 3-nitrophenol based up its Henry's Law constant of 2 x 10^{-9} atm-m^3/mole (25ºC). Similarly, from its Henry's Law constant, 3-nitrophenol should not volatize from moist soil to the ambient atmosphere (Gaffney et al. 1987; Barley and McFiggins 2010).

Based on its vapor pressure of $1.5x10^{-4}$ mm Hg at 25°C (Table 1), 3-nitrophenol should not volatize from dry soil surfaces to any appreciable extent, and should exist exclusively as a vapor in the ambient atmosphere. 3-nitrophenol completely biodegraded in aqueous soil media within 5 days (Haller 1978). Several studies strongly suggest that an important environmental fate pathway for 3-nitrophenol is the readily biodegradable nature of this compound in water columns. However, it took more than 64 days for the compound to biodegrade in soil (Alexander and Lustigman 1966).

Table 1. Summary of Physical-Chemical Properties of Nitrophenol

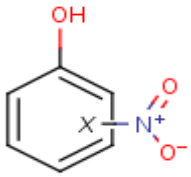
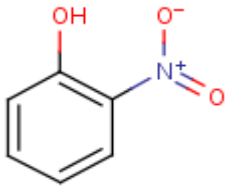
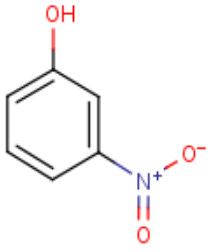
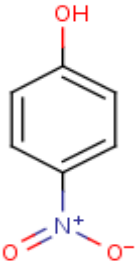
Structure				
	Nitrophenols	**2-Nitrophenol**	**3-Nitrophenol**	**4-Nitrophenol**
CAS No.	25154-55-6	88-75-5	554-84-7	100-02-7
Molecular weight[1]	139.109	139.109	139.109	139.109
Color[1]	Colorless to slightly yellow	Light yellow	Colorless to yellow	Colorless to yellow
Physical State[1]	Crystals	Needles/prisms	Crystals	Crystals
Melting Point[1]	NA	44-45°C	96.8°C	113-114°C
Boiling Point (760 mmHg)[1]	NA	216°C	NA	279°C
Odor[1]	Odorless to peculiarly aromatic	Peculiarly aromatic	NA	Odorless
Solubility in water (25°C)[1]	Slightly	2.5 g/L	13.6 g/L	15.6 g/L
Solubility in other solvents[1]	Soluble in alcohol, ether	Very soluble in alcohol, ether, acetone, chlorine	Very soluble in ethanol, ether, and acetone	Freely soluble in alcohol, chloroform, ether
Partition Coefficients				
Log K_{ow} [1]	NA	1.79	2.00	1.91
Log K_{oc}[1]	NA	1.1-2.4	1.7-2.5	1.2-2.7
Vapor pressure at 25°C[1]	NA	1 x 10^{-1} mmHg	1.5 x 10^{-4} mmHg	5 x 10^{-4} mmHg
Henry's Law constant [1]	NA	1.63 x 10^{-5} atm-m^3/mole (25°C)	2 x 10^{-9} atm-m^3/mole (25°C)	1.28 x 10^{-8} atm-m^3/mole (20°C)
Vapor density	NA	NA	NA	NA
Conversion Factors	1 mg/m^3 = 0.173 ppm 1 ppm = 5.78 mg/m^3			

Table 1 Legend:
^{o}C = degrees Celsius
NA = Not Available
g/L = grams per liter
mmHg = millimeters mercury
mg/m^3 = milligrams per cubic meter
m^3/mole = cubic meters per mole
atm-m^3/mole = cubic meters of atmosphere per mole
ppm = parts per million
Log Kow = octanol-water partition coefficient
Log Koc = organic carbon partition coefficient

Notes:
[1]HSDB 2013a–d; [2]WHO 2000

Available data for 4-nitrophenol informs that it can be released into the environment via a number of waste water columns during its manufacture and used in the production of dyestuffs and compounds that are used to treat leather, such as methyl and ethyl parathion, and N-acetyl-p-aminophenol (acetaminophen) (HSDB 2013d). Additionally, 4-nitrophenol might potentially form in the ambient atmosphere following photooxidation of nitrobenzene, and from aromatic hydrocarbons such as benzene, toluene, and phenanthrene with nitric oxide in the air. On release to the ambient atmosphere, 4-nitrophenol will exist in both particulate and vapor phases based on its vapor pressure of 9.79 x 10^{-5} mm Hg at 20^{o}C or 5 x 10^{-4} mm Hg at 25^{o}C (Table 1). Vapor-phase 4-nitrophenol will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 3.7 days.

Particulate 4-nitrophenol is removed from the air by wet and dry deposition. Vapor 4-nitrophenol is removed from the ambient air through dissolution into cloud water with subsequent precipitation. The log K_{OC} value of 4-nitrophenol is 1.2–2.7 (i.e., > 500), and based on these values (Xu et al. 2001; Løkke et al. 1984), 4-nitrophenol is expected to display very high to low mobility. However, a reported median log K_{OC} of 2.37 suggests that this compound is only moderately mobile in the soil substrate (Schüürmann et al. 2006). Further, volatilization from moist (Tremp et al. 1993) or dry soil surfaces (Schwarzenbach, et al. 1988), or from water surfaces, is not expected to occur or to represent an important fate process based upon its Henry's Law constant of 1.28 x 10^{-8} atm-m^3/mole at 20^{o}C and vapor pressure of 5 x 10^{-4} mm Hg, respectively (HSDB 2013d). The biodegradation half-life of 4-nitrophenol in an acidic soil environment was reported as 2.5 days, and in basic soil the biodegradation half-life of 4-nitrophenol was reported as 10.2 days. Based on its K_{OC} value, it is possible that on release to water columns, 4-nitrophenol might adsorb to suspended solids and sediment, at least to some extent. The biodegradation half-life of 4-nitrophenol was reported as 18 hours and 6.8 days in aerobic and anaerobic waters, respectively (Capel and Larson 1995; Nyholm et al. 1984).

In summary, release of nitrophenol isomers into the environment is primarily mediated by emissions into the air, water, and soil from highly diffuse origins such as gasoline and diesel-engine vehicles, hydrolytic and photolytic degradation of a number of organophosphorus pesticides, and diverse medical and industrial products. Additional releases into the hydro- and geosphere are caused by the dry and wet deposition of airborne nitrophenols from the

atmosphere. According to current research data, only a slow rate of volatilization of 2-nitrophenol is to be expected, while very little or no significant volatilization of 3- or 4-nitrophenol from water to air is to be expected. Whereas 2-nitrophenol is enriched in the liquid phase of clouds, physicochemical data shows that comparatively higher levels of 3-nitrophenol and 4-nitrophenol than expected can be found in the gas phase as a result of extensive binding to particulate matter.

Due to their inherent water solubility and expected occurrence in the vapor phase, wet deposition of mono-nitrophenols from the atmosphere to surface water columns and soil is expected. Following its emission to the troposphere, the major transformation pathway for 2-nitrophenol is expected to be rapid nitration to 2,4-dinitrophenol. By contrast, the majority of atmospheric 3- and 4-nitrophenol should be particle-bound, and thus both isomers will only be minimally available for photochemical reactions. Much of the 3- and 4-nitrophenol should be "washed out" from the air by wet and dry deposition. It is unlikely that mono-nitrophenols contribute directly to the depletion of the stratospheric ozone layers or to global warming. Moreover, several studies that have focused on nitrophenol isomer degradation suggest that 2- and 4-nitrophenol are readily biodegradable in water under aerobic conditions, and mineralization of nitrophenols under anaerobic conditions requires extended adaptation of microbial communities (ATSDR 1992).

Furthermore, measured K_{OC} values in the range of 13 to greater than 500 indicate a low to moderate potential for soil adsorption. Nitrophenols released to the soil are likely to undergo biodecomposition under aerobic conditions. Infiltration into groundwater is expected only under conditions that are unfavorable to biodegradation. For 2- and 4-nitrophenol, measured bioconcentration factors (BCF) ranging from 11 to 76 indicate a low potential for bioaccumulation (Eichenbaum et al. 2009).

2.3 Bioaccumulation and Elimination

All isomers of mono-nitrophenols are expected to display a low potential for bioaccumulation. In aquatic test system, BCFs that ranged from 14.6 to 24.4 were estimated for 2-nitrophenol in a semi-static zebrafish (*Brachydanio rerio*) test system (Koerdl et al. 1981). Additionally, in a flow-through experiment, estimated BCFs ranged from 30 to 76 for the common carp (*Cyprinus carpio*), including possible conjugates (Broecker et al. 1984). An experimental BCF of 25 in fish for 3-nitrophenol was previously reported (Sacan et al. 2004), while an estimated BCF of 10 was calculated in fish using a log K_{OW} of 2.0 and regression analysis (Hansch et al. 1995; U.S. EPA, 2012). Similar to 2-nitrophenol, the BCF values for 3-nitrophenol also suggest that bioconcentration in aquatic organisms is low (Franke et al. 1994).

In the context of 4-nitrophenol, static test analyses showed that accumulation factors of 11 were determined for the green alga *Chlorella fusca* after 1 day (Geyer et al. 1981), and accumulation factors of 57 were determined for the freshwater golden orfe (*Leuciscus idus melanotus*) after 3 days of exposure (Freitag et al. 1982). Zebrafish exposed in tap and river water nearly completely eliminated the accumulated ^{14}C-4-nitrophenol within 48 hours (Ensenbach and Nagel 1991). Starfish (*Pisaster ochraceus*) and sea urchin (*Strongylocentrotus purpuratus*)

eliminated 89% and 36%, respectively, of injected ^{14}C-4-nitrophenol (3.48 and 3.70 mg/kg body weight, respectively) within 8 hours (Landrum and Crosby 1981).

Studies in animals have yielded only limited information for 2-nitrophenol. For example, in rabbits given a single dose of 200–330 milligrams per kilograms (mg/kg) body weight via oral gavage, the majority of the administered dose (approximately 80%) was excreted via the urine within 24 hours of dosing. Further, approximately 71% was conjugated with glucuronic acid, and approximately 11% with sulfate. By contrast, only about 3% was reduced to aminophenols (Robinson et al. 1951). Additionally, the skin permeability of 2-nitrophenol was shown in several *in vitro* studies (Huq et al. 1986; Jetzer et al. 1986; Ohkura et al. 1990). Although the available information is somewhat limited, the bioaccumulation of 2-nitrophenol in organisms is not to be expected due to its demonstrated rapid metabolism and excretion. After oral, dermal, intravenous, or intraperitoneal dosing of 4-nitrophenol to several test species (i.e., rats, mice, dogs, or rabbits), most of the applied dose (up to 95%) was excreted as glucuronide and sulfate conjugates of 4-nitrophenol via the urine, and within 24–48 hours of dosing the animals. In addition, only negligible quantities were excreted via feces (about 1%) or as unchanged 4-nitrophenol (about 2–7%). The percent occurrence of glucuronide and sulfate conjugates was found to be species-, gender-, and dose-dependent. Moreover, although sulfate conjugation dominates at lower concentrations of 4-nitrophenol, the percentage of glucuronide conjugates increases at higher doses of administered 4-nitrophenol (Robinson et al. 1951; Gessner and Hamada 1970; Machida et al. 1982; Rush et al. 1983; Snodgrass 1983; Tremaine et al. 1984; Meerman et al. 1987).

In rabbits, it was also shown that after oral dosing, 4-nitrophenol is reduced to 4-aminophenol and is subjected to glucuronidation and sulfation. In addition, up to 14% of the administered dose of 4-nitrophenol was detected as amino compounds in the urine (Robinson et al. 1951). After intraperitoneal dosing in mice, 4-nitrophenyl glucoside was identified as a minor metabolite of 4-nitrophenol (i.e., at approximately 1–2% of the administered dose; Gessner and Hamada 1970). In the context of 4-nitrophenol, the pretreatment of laboratory animals with ethanol (i.e., to induce functional expression of cytochrome P-450) resulted in a marked increase in hepatic microsomal hydroxylation. Subsequently, the resultant formation of 4-nitrocatechol competed with 4-nitrophenol for the glucuronidation and sulfation pathways (Reinke and Moyer 1985; Koop 1986; McCoy and Koop 1988; Koop and Laethem 1992).

Studies on dermal resorption under non-occlusive conditions showed dermal uptake of about 35% and 11% of the applied dose of ^{14}C-4-nitrophenol within 7 days in rabbits and dogs, respectively. Skin penetration of 4-nitrophenol was also shown in several *in vitro* studies (Huq et al. 1986; Jetzer et al. 1986; Ohkura et al. 1990). Rapid metabolism and excretion make bioaccumulation of 4-nitrophenol in organisms unlikely. Irrespective of whether exposure was via the dermal or oral administration routes, other researchers have found that 4-nitrophenol was eliminated rapidly, and done so primarily via the urine. For example, following a single dermal dose (160 mg/kg) of 4-nitrophenol in male and female mice, the rate of absorption was rapid as determined from the time at which maximum concentration of the substance was achieved in the plasma following dose administration (t_{max}) values of 1 and 2 hours, respectively. Further, elimination of 4-nitrophenol was quenched, at least in part, by prolonged dermal absorption that generated terminal half-life values of 4.31 and 4.92 hours, respectively

(Eichenbaum et al. 2009). The estimated absolute bioavailability after dermal dosing (F_{dermal}) was 21% and 19% in male and female mice, respectively (Eichenbaum et al. 2009). Female rats were treated dermally with 5-micrograms (µg) 4-nitrophenol/rat. Within 4 hours of initiating treatment, 30–40% of the administered dose was eliminated in the urine, which increased to 60–70% urine elimination after 24 hours. After 120 hours, almost none of the 4-nitrophenol remained in the untreated skin, and less than 1% of the compound remained in the treated skin (Eichenbaum et al. 2009).

Further analyses revealed that about 30% of the compound was washed off after 24 hours of application. At 120 hours following initiation of the treatment, it was found that less than 1% remained in the body; 66% was eliminated in the urine and 3% was eliminated in the feces of rats (Hughes and Hall 1997). Following oral dosing for 3 days with 25 µg/day of 4-nitrophenol, 8% of the compound was eliminated in the feces and 71% in the urine. None of the administered 4-nitrophenol was detected in either the adipose or lung tissues. Very little of the administered 4-nitrophenol (i.e., less than 0.02%), was retained in the liver and approximately 0.4% was retained in the carcass (Freitag et al. 1982).

2.4 Summary of Mammalian Toxicity

2.4.1 Mammalian Oral Toxicity—Acute

Acute toxicity studies indicated a wide range of LD_{50} values in both rats and mice (summarized in Table 2). For 2-nitrophenol, the oral LD_{50} value was in the range of 2830–5376 mg/kg body weight in rats (BASF 1970 – as cited in Boehncke et al. 2000; Vernot et al. 1977; Koerdel et al. 1981) and 1300–2080 mg/kg body weight in mice (Vasilenko et al. 1976; Vernot et al. 1977). Clinical signs following oral exposure were non-specific and included observations of dyspnea, staggering, trembling, somnolence, apathy, and cramps. The macroscopic examination that was performed in some studies revealed congestion in the liver and kidneys and ulcers of the stomach in high-dose treated rats. In addition, in cats (two animals per dosed group), the oral application of 2-nitrophenol (50, 100, or 250 mg/kg body weight; no controls) resulted in a dose-dependent increase in methemoglobin (6%, 44%, and 57% at low, moderate and high doses, respectively). One animal died following administration of 2-nitrophenol at a dose of 250 mg/kg body weight.

For 3-nitrophenol, the dose expected to result in 50% lethality (LD_{50}) value for oral exposure of the male Sprague-Dawley rat was 930 (range 640–1350) mg/kg (Vernot et al. 1977). From a related study, the LD_{50} value for oral exposure was determined to be 328 mg/kg in rats (Lewis 2004). In mice, the oral LD_{50} value was determined to be 1070 mg/kg (Lewis 2004).

In studies submitted to the U.S. EPA (1998) for pesticide registration of 4-nitrophenol, the oral LD_{50} in dosed rats was reported as 191 mg/kg in males and 170 mg/kg in female rats. Clinical signs seen in the acute oral toxicity study cited above included ataxia, convulsions, and diarrhea at a dose of 200 mg/kg or higher in female rats; however, observed signs did not show any apparent dose-response relationship. A second study submitted to the U.S. EPA (Branch 1983) reported that the oral LD_{50} of 4-nitrophenol was 263 mg/kg in male rats and 202 mg/kg in female rats. In addition, based on observations of lethargy and dyspnea in both male and female rats, a LOAEL of 70 mg/kg was reported, which was the lowest dose tested. Clinical abnormalities that

were usually followed by death included convulsions, prostration, and dyspnea. At least one of these signs of intoxication occurred in 16 rats on the day of dosing, following which 15 of these animals died on the same day they received their first dosing. In animals that survived to termination, the predominant or exclusively observed clinical effects included lethargy, ptosis (palpebral), salivation, and bright yellow coloration of the passed urine. Each effect occurred in at least 10 rats. In addition, tremors were observed in three animals, two of which survived for the duration of the study.

The combined oral LD_{50} value of 4-nitrophenol for male and female rats was 350 mg/kg. Some animals were prostrate within 5 minutes of being dosed. In addition, breathing was shallow, and this clinical manifestation was followed by the onset of coma. At autopsy, the yellow color of the 4-nitrophenol test material was present in the liver and kidneys. There was considerable pulmonary congestion and moderate liver congestion. The gastric mucosa showed slight inflammation (Younger Laboratories 1956).

This study did not report any doses that corresponded to pathology or clinical signs. The oral LD_{50} of 4-nitrophenol was in the range of 220–620 mg/kg body weight in rats (Boehncke et al. 2000). In acute oral toxicity studies for 4-nitrophenol in rats, clinical signs following oral exposure were non-specific and included tachypnea and cramps. Further, the macroscopic examination performed in some studies revealed a greyish discoloration with dark red patches of the lungs (Vernot et al. 1977; Boehncke et al. 2000). Cats dosed at 100, 200, or 500 mg/kg at single dose of 4-nitrophenol showed mortality 0/2, 1/2, 2/2 respectively and no formation of methemoglobin formation (BASF, 1969 – as cited in Boehncke et al. 2000).

Table 2. Summary of Acute Oral Toxicity for Nitrophenols in Mammals

Test Organism	LD_{50} (mg/kg)	Test Results			Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Rats (male)	263	<70	70	Lethargy and dyspnea	Branch 1983 (4-nitrophenol)
Rats (female)	202	<70	70	Lethargy and dyspnea	Branch 1983 (4-nitrophenol)
Rats (sex not stated)	202–616	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Rats (female)	220–620	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Rats (male)	620	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Rats (male)	191	NA	NA	None stated	U.S. EPA 1998 (4-nitrophenol)
Rats (female)	170	NA	NA	None stated	U.S. EPA 1998 (4-nitrophenol)
Rats (sex not stated)	620 (450–850)	NA	NA	None stated	Vernot et al. 1977 (4-nitrophenol)
Rats (both sexes)	350	300	350	Increased pulmonary congestion and moderate liver congestion	Younger Labs 1956 (4-nitrophenol)
Rats (sex not stated)	2830 (2050–3890)	NA	NA	None stated	Vernot et al. 1977 (2-nitrophenol)
Rats (sex not stated)	930 (640–1350)	NA	NA	None stated	Vernot et al. 1977 (3-nitrophenol)
Rats (sex not stated)	328	NA	NA	None sated	Lewis 2004
Rats	50	NA	NA	None stated	U.S. EPA 1992 (4-nitrophenol)
Mice (sex not stated)	380–467	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Mice (both sexes)	40	NA	NA	Mortality	NTP 1993
Mice (male)	470	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Mice (female)	626	250	500	Mortality	Plasterer et al. 1985 (4-nitrophenol)
Mice (sex not stated)	470 (320–690)	NA	NA	None stated	Vernot et al. 1977 (4-nitrophenol)
Mice (sex not stated)	1300 (890–1700)	NA	NA	None stated	Vernot et al. 1977 (2-nitrophenol)
Mice (sex not stated)	1410 (No CL)	NA	NA	None stated	Vernot et al. 1977 (3-nitrophenol)
Mice (sex not stated)	1070	NA	NA	None stated	Lewis 2004
Cats (sex not stated)	200 mg/kg (50% mortality	NA	NA	No increase in methemoglobin formation (4-nitrophenol)	BASF 1969

Legend:
mg/kg = milligram per kilogram
mg/kg-day = milligram per kilogram per day

Table 2 Legend (continued):
NOAEL= no observed adverse effect level
LOAEL = lowest observed adverse effect level
LD_{50} = dose expected to result in 50% lethality
NA = not applicable

The oral LD_{50} of 4-nitrophenol administration to rats was previously determined to be in the range of 220–620 mg/kg body weight (BASF, 1969 – as cited in Boehncke et al. 2000; Vasilenko et al. 1976; Hoechst, 1977a; Vernot et al. 1977; Andrae et al. 1981). By contrast, when administered to mice, the oral LD_{50} of 4-nitrophenol was determined to be in the range of 380–470 mg/kg body weight (Vasilenko et al. 1976; Vernot et al. 1977). Clinical signs following oral exposure of rats were non-specific and included tachypnea and cramps. In female mice, the LD_{50} for 4-nitrophenol was 625.7 mg/kg with a NOAEL of 250 mg/kg and a LOAEL of 500 mg/kg based on observations of mortality. No effects on body weight gains were observed at doses of 4-nitrophenol up to 1,000 mg/kg (Plasterer et al. 1985).

2.4.2 Mammalian Oral Toxicity—Subchronic

In a previously published study, the effect of 2-nitrophenol in rats was explored in a 28-day study to evaluate the Organization for Economic Co-operation and Development (OECD) Test Guideline 407 (Koerdel et al. 1981). In this study, five animals per gender per dosed group were exposed to daily doses of 0, 22, 67, or 200 mg/kg body weight via oral gavage. Following exposure to 2-nitrophenol, high-dose males and mid- and high-dose females displayed decreased food intake. Final body weights decreased in all dosed animals, but this was not statistically significant. In addition, absolute liver and kidney weights decreased in mid-dose animals. Relative weights of the testes also increased in the low- and mid-dose male rats; however, in male rats given high doses of 2-nitrophenol, the weights of the testes increased. In all dosed rats, relative and absolute weights of the adrenal glands increased. Further studies of hematological, clinical chemistry, and histopathological examination of the major organs and tissues failed to indicate adequately any evidence of a substance-related toxic effect in comparison with the controls (Koerdel et al. 1981). Moreover, due to poor record-keeping and the fact that only relatively minor effects (i.e., weight of adrenal glands) were shown by all exposed animals, a reliable NOAEL could not be accurately determined (Koerdel et al. 1981).

In a 28-day study conducted to evaluate OECD Test Guideline 407, Sprague-Dawley rats (at 10 animals per gender per dosed group) received daily doses of 0, 70, 210, or 630 mg 4-nitrophenol/kg body weight via oral gavage (Andrae et al. 1981). After dosing the animals, impaired locomotive abilities were observed for approximately 2 hours in animals exposed to 4-nitrophenol at the mid- and high-dose levels. In the mid-dose animals, 1 male rat (i.e., 10% of the group) died; in the high-dose male and female rats, the mortality rate was 4/10 and 6/10 (i.e., 40% and 60%), respectively. However, the specific indicators or observations of *in vivo* toxicity in these bioassays were not given. In the lowest dose group, the macroscopic examination of the animals revealed seven cases of pale liver, and histopathological examination showed 14 cases of finely dispersed fatty degeneration. A focal fatty degeneration of the liver was also seen in 13/20 (i.e., 65%) of the mid-dose treated rat group, but not in animals treated with high-dose 4-nitrophenol (Andrae et al. 1981).

However, it was reported that finely dispersed fatty degeneration was also seen in 6/20 (i.e., 30%) of the control animals. A swelling of the liver cells (i.e., a hydropic change) was noted in 4/10 (i.e., 40%) of the high-dose, 4-nitrophenol treated male rats, although this was not seen in female rats (Andrae et al. 1981). All high-dose rats that died before the end of the study showed vascular congestion of the liver. At 4-nitrophenol doses of 210 and 630 mg/kg body weight, a slight increase in leukocyte count was seen in male and female rats. The increase in leukocyte count was significant in the high-dose treated female rats. In high-dose treated male rats, the activity of alanine aminotransferase was also significantly increased. Other substance-related effects in high-dose 4-nitrophenol treated animals included increased nephrosis (in two male and five female rats), testicular atrophy (observed in one male rat) and inhibition of spermatogenesis (observed in two male rats), and ovarian follicular atresia (observed in four female rats). Since exposure to 4-nitrophenol produced largely unclear effects in the liver, a NOAEL could not be determined from this data (Andrae et al. 1981).

In newborn rats that had been dosed orally for 18 days beginning when rats were age 4 days, there was no mortality seen in animals that had been dosed with 4-nitrophenol at 110 milligrams per kilogram per day (mg/kg-day); however, mortality was observed at doses of 160 mg/kg-day and higher. No clinical signs or other adverse effects were observed at any dose (Koizumi et al. 2001). Following 4-nitrophenol treatment for 28 days that began when rats attained an age of 6 weeks, it was noted that 10 of the 12 male and 10 of the 12 female rats died following treatment with 1,000 mg/kg-day 4-nitrophenol after animals showed evidence of oligopnea and were lying in a prone/lateral position. The only observed pathology was a higher incidence of eosinophilic bodies in kidney proximal tubular cells in male rats treated with 400 and 1,000 mg/kg-day 4-nitrophenol. No other clinical or adverse effects were observed (Koizumi et al. 2001).

In a subchronic toxicity study, 4-nitrophenol was administered to rats (20 rats per group) for 13 weeks in water by oral gavage at doses of 0, 25, 70, or 140 mg/kg-day. At 70 mg/kg-day, one male and one female rat died and the remaining surviving female rats displayed increased incidence of urine staining between weeks 7 to 9. At 140 mg/kg-day, 14 male and 6 female rats died and there was urine staining in females (Schulze, 1989b). Observed clinical signs preceding death included the following outcomes: pallor, wheezing, dyspnea, prostration and languid behavior. Death of one female rat following a dose of 25 mg/kg-day was unlikely treatment-related. No compound-related effects were found for changes in body weight, food consumption, hematology, clinical chemistry or when examining organ weights. Thus, based on observations of an increased acute mortality incidence, and the associated clinical signs and pathology with possible urine staining in female rats, the LOAEL was 70 mg/kg-day and the NOAEL was 25 mg/kg-day (Schulze, 1989b; U.S. EPA, 1998).

2.4.2.1 Mammalian Oral Toxicity—Subchronic: Developmental Toxicity

No studies reporting chronic effects from oral exposure were found for review, and thus subchronic studies were considered for developmental effects.

In a range-finding study, pregnant rats (five dams per group) were treated with 2-nitrophenol at doses of 0, 50, 125, 250, or 1,000 mg/kg body weight by oral gavage from day of gestation (GD) 6–15 and uterine examination on GD20. Administration of 2-nitrophenol at doses of 500 and

1,000 mg/kg caused signs of maternal toxicity in dosed rats (IRDC, 1983). One mortality was observed—although the precise cause of death could not be determined. Excessive salivation occurred in two rats in the highest dose group of 1,000 mg/kg-day. Darkly stained urine occurred in rats that were dosed with 250, 500, and 1,000 mg/kg-day. Other observations observed in animals dosed at 250 mg/kg-day included yellow staining of the hair coat at the nose, mouth, and anogenital area.

Table 3. Summary of Subchronic Oral Toxicity for Nitrophenols in Mammals

		Test Results			
Test Organism	**Test Duration**	**NOAEL (mg/kg/day)**	**LOAEL (mg/kg/day)**	**Effects Observed at the LOAEL**	**Study**
Newborn rats (both sexes)	18 days	110	230	No clinical signs observed. LOAEL based on mortality	Koizumi et al. 2001 4-nitrophenol
Rats (male)	28 days	160	400	Higher incidence of eosinophilic bodies in proximal tubular cells of the kidneys	Koizumi et al. 2001 4-nitrophenol
Rats (female)	28 days	400	1,000	Mortality after showing oligopnea and lying in a prone/lateral position	Koizumi et al. 2001 4-nitrophenol
Rats (both sexes)	28 days	100	>100	No adverse effects reported	Schulze 1989a 4-nitrophenol
Rats (both sexes)	13 weeks	25	70	Acute mortality and wheezing, dyspnea, pallor, prostration and languid behavior	Schulze 1989b 4-nitrophenol
Rats (sex not reported)	13 weeks	25	70	3/13 mortality	Schulze 1989b 4-nitrophenol
Mice (female)	GD7–14	<400	400	Maternal mortality	Plasterer et al. 1985 4-nitrophenol
Rats (female)	GD11	333	667	Maternal mortality	Kavlock 1990 4-nitrophenol
Rats (female)	GD6–16	13.8	27.6	Maternal weight gain	U.S. EPA 1998 4-nitrophenol
Rats (female)	GD6–15	125	250	Maternal toxicity: darkly stained urine	IRDC 1983 2-nitrophenol

Legend:
mg/kg = milligram per kilogram
mg/kg/day = milligram per kilogram per day
NOAEL= no observed adverse effect level
LOAEL = lowest observed adverse effect level
GD = gestation day

Body weight was reduced in the 500 and 1,000 mg/kg groups early in the treatment period; however, these animals recovered. In addition, at the highest 2-nitrophenol dose level of 1,000 mg/kg body weight, a slight but statistically significant (compared with historical controls) increase in group mean post-implantation losses (i.e., 13.8% versus 8.2% in controls) and mean early resorptions (i.e., 2.3% versus 1.2% in controls) was seen. No treatment-related findings at necropsy or in the number of viable fetuses, implantations, or corpora lutea were observed (IRDC 1983).

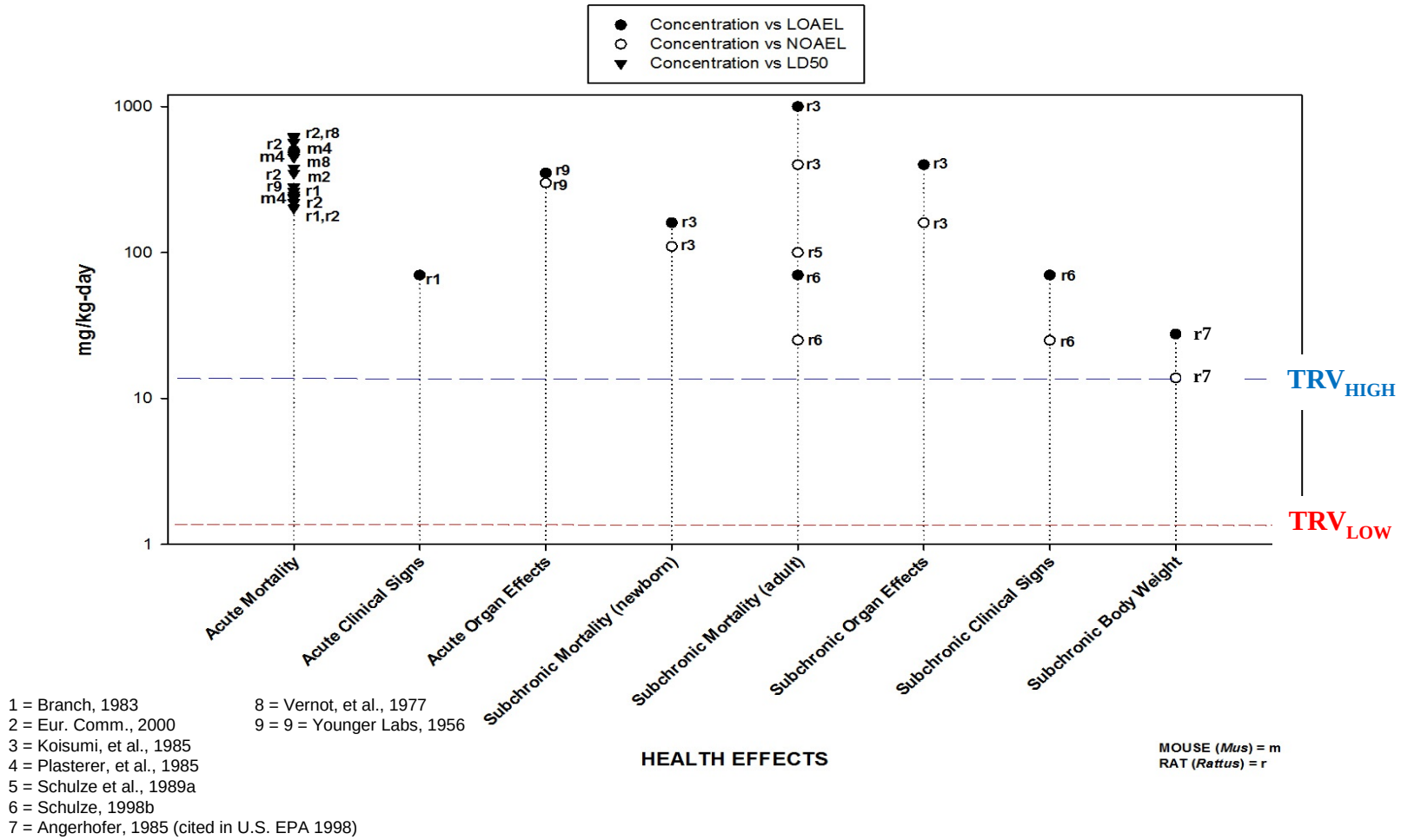
In two previously reported studies of the effects of 4-nitrophenol (Hardin et al. 1987; Kavlock 1990), there were no examinations of the pups for possible teratogenic manifestations. Both studies also had inherent limitations that included the use of only one dose group or an exposure to a mixture of compounds. For that reason, reliable NOAEL values were not derived.

In the first study (Branch et al. 1983), groups of 50 female CD-1 mice were treated with daily oral gavage administered doses of 400 mg 4-nitrophenol/kg body weight from GD7-14. The rate of survival in pregnant mice (n = 36) was 81 percent as compared 100 percent survival in controls (Branch et al. 1983). In addition, dosed animals showed decreases in maternal weight gain, and no changes were observed in the reproductive index (i.e., ratio between surviving offspring and surviving pregnant mice). The mean number of live pups per litter had decreased slightly, but treatment of animals with 4-nitrophenol did not provoke any gross abnormalities.

In the second study (Kavlock 1990), the developmental toxicity of 4-nitrophenol was explored when administered by oral gavage to Sprague-Dawley rats. In this study, 4-nitrophenol was dissolved in a mixture of water, Tween 20, propylene glycol, and ethanol at a 4:4:1:1 ratio. After this, groups of 12–13 rats were treated with 0, 100, 333, 667, or 1,000 mg/kg body weight on GD11. Measured end-points of maternal toxicity included signs of toxicity, mortality, gain in body weight, and the number of implantation scars in the uterus of each rat at weaning. In the pups, viability, body weight on post-natal days 1–6, clear evidence of malformations, and perinatal losses were measured. At a dose of 667 mg/kg body weight, the mortality of dams had increased. At a dose of 333 mg/kg, body weight on post-natal days 1–6 was unaffected. In addition, the litter size on post-natal days 1–6 was non-significantly reduced (Kavlock 1990).

In a developmental toxicity study that was submitted to the U.S. EPA (1998), 4-nitrophenol was administered by oral gavage to pre-mated female rats in propylene glycol, at dose levels of 0, 1.4, 13.8, or 27.6 mg/kg-day from GD 6–16. At 27.6 mg/kg-day, there was evidence of decreased maternal body weight and weight gain during the dosing period. No treatment-related effects on mortality, clinical signs, food consumption, or cesarean parameters were reported. Based on body weight loss or gain, a maternal LOAEL of 27.6 mg/kg-day was derived, and the maternal NOAEL was determined to be 13.8 mg/kg-day.

Treatment-related developmental toxicity was also observed. However, the small number of litters (n = 10) that were available for examination at the high dose of 4-nitrophenol treatment, along with a lack of some key experimental details, collectively served to compromise interpretation of the results. From this study, the developmental NOAEL was tentatively derived as 27.6 mg/kg-day, while a developmental LOAEL was not established from the observations made in this study (U.S. EPA 1998).



1 = Branch, 1983
2 = Eur. Comm., 2000
3 = Koisumi, et al., 1985
4 = Plasterer, et al., 1985
5 = Schulze et al., 1989a
6 = Schulze, 1998b
7 = Angerhofer, 1985 (cited in U.S. EPA 1998)
8 = Vernot, et al., 1977
9 = 9 = Younger Labs, 1956

Figure 1. 4-Nitrophenol Oral Ingestion Health Effects to Mammals

Legend:
TRV = toxicity reference value
Note: UF of 10 was applied to the subchronic results to arrive at the TRVs shown here.

In two other previously published studies (Plasterer et al. 1985; Hardin et al. 1987), it was shown that a dose of 400 mg/kg-day 4-nitrophenol did not cause any developmental effects (i.e., at the maximal tolerated dose). Changes in maternal weight of dosed animals were less than those observed among controls (Plaster et al. 1985). The percentage of viable litters was also unaffected. In addition, no differences were seen in the number of pups per litter or percentage of pup survival compared to controls. Further, there were no differences in the birth weight or the weight gain of exposed pups compared to the controls (Plasterer et al. 1985; Hardin et al. 1987).

2.4.3 Mammalian Oral Toxicity—Chronic

No studies reporting chronic effects from oral exposure were found for review.

2.4.4 Mammalian Inhalation Toxicity

2.4.4.1 Mammalian Inhalation Toxicity—Acute

The inhalation exposure of rats to an atmosphere that was saturated with 2-nitrophenol at 20°C for 8 hours (no further information was available in the original manuscript) did not result in any mortality and there were no signs of toxicity (BASF 1970 – as cited in Boehncke et al. 2000).

Some studies reported observations from gross necropsy and macroscopic examination, which revealed a greyish discoloration with dark red patches of the lungs. After a 14-day observation period, no mortality was seen in rats following a single 4-hour head-only exposure to the sodium salt dust of 4-nitrophenol at 4,700 milligrams per cubic meter (mg/m^3) (although the particle size was not provided). In four of the six dosed rats, the appearance of corneal opacity was observed at the end of the exposure, which persisted through the entire observation period of 14 days. In two additional rats that were exposed to 1,510 mg/m^3, the methemoglobin concentrations remained unchanged as compared with the controls. However, a determination of methemoglobin concentrations after exposure to 4-nitrophenol 4,700 mg/m^3 was not performed (Smith et al. 1988). No other clinical signs were seen, other than transient weight loss and corneal opacity at 4.7 mg/L (Smith et al. 1988). Another document reported an LC_{50} of > 4.7 mg/L for 4-nitrophenol with no reported clinical signs (Eur. Com. 2000).

In another inhalation study, rats were exposed to a 4-nitrophenol saturated atmosphere at 20°C for 8 hours (BASF 1969 – as cited in Boehncke et al. 2000). At the end of the exposures, no mortality and no signs of toxicity were seen in any of the animals (BASF 1969 – as cited in Boehncke et al. 2000). From additional studies that were conducted in both rats and guinea-pigs, the dermal LD_{50} was identified as 1,000 mg/kg body weight (Hoechst 1977b; Andrae et al. 1981).

2.4.4.2 Mammalian Inhalation Toxicity—Subchronic

In Sprague-Dawley rats (n = 15 per gender per group), no mortality was observed after exposure to melted 2-nitrophenol vapors at concentrations of 0, 5, 30, or 60 mg/m^3 (i.e., a "whole body" exposure) for 6 hours/day, and 5 days/week, over a period of 4 weeks. With the noted exception of squamous metaplasia of the epithelial lining of the maxilloturbinates and

nasoturbinates in all of the rats exposed to 60 mg/m^3, clinical and histopathological examinations failed to demonstrate consistent exposure-related effects. Methemoglobin values that were determined after the eleventh exposure to 2-nitrophenol vapors were significantly increased only in animals exposed to low-dose 2-nitrophenol (males: 1.0, 2.3, 1.8, and 1.6%; females: 2.0, 4.1, 2.1, and 1.1%). However, these values were within the observed range of control values at the end of the study (Hazleton Lab. 1984).

After ten 6-hour exposures of 6-week-old male rats to the dust of the sodium salt of 4-nitrophenol (in two separate tests; at 0.3 and 2.5 mg/L, and 0.01 and 0.1 mg/L), investigators reported no clinical signs of irritation in any of the test groups (Coate 1983). In this study, rats were treated for 5-continuous days, with 2 days off from the exposure, which was then followed by a repeated exposure to the 4-nitrophenol dust for an additional 5 days. After 10 exposures, methemoglobinemia was seen in all of the treated rats. Additionally, darker urine and proteinuria were seen in both test groups. The 2.5 mg/L exposure group showed reduced urine volume and, after 10 exposures, the 2.5 mg/L test rats showed a mean absolute spleen weight that was significantly lower than that of the control group. Following the tenth exposure, rats exposed to 0.1 mg/L 4-nitrophenol exhibited elevated methemoglobin levels. Fourteen days later, methemoglobin levels returned to normal in rats that were exposed to 0.1 mg/L. After a14-day recovery period, the 2.5 mg/L exposure group showed elevated numbers of erythrocytes, hemoglobin levels, hematocrit, and levels of serum glutamic-oxalacetic transaminase (SGOT) and creatinine. The SGOT levels were also elevated in the 0.3 mg/L exposure group. After 14 days following exposure at 2.5 mg/L, two of the five exposed rats exhibited sustained but elevated levels of methemoglobin, and the 2.5 mg/L test rats showed mean absolute and relative spleen and lung weights that were significantly lower than were found for the controls (Smith et al*.* 1988). No adverse effects were seen following a 4-week inhalation study in which rats were exposed up to 0.03 mg/L of the 4-nitrophenol dust. The only clinical sign that was observed in these animals was cataracts and corneal keratitis sicca in some of the male and female rats that had been exposed to 0.03 mg/L (Coate 1983).

Subchronic inhalation exposures of rats to 4-nitrophenol have been previously reported by several groups (Smith et al. 1988; Coate 1983; Hazleton Lab 1983). In one study (Smith et al. 1988), no mortality was observed after “head-only” exposure of male albino Crl:CDR rats (n = 10 per group) to the dust of the sodium salt of 4-nitrophenol at doses of 0, 340, or 2,470 mg nitrophenol dust/m^3 (mass median aerodynamic diameter [MMAD] 4.6–7.5 µm). In this study, rats were exposed for 6 hours/day, and 5 days/week, over a period of 2 weeks. Both exposure concentrations of 4-nitrophenol provoked irritation in treated rats. Additionally, after exposure to 340 and 2,470 mg/m^3, there was evidence of darker urine and proteinuria in treated rats, as well as elevated levels of aspartate aminotransferase. Exposed mice also exhibited a dose-dependent increase in methemoglobin levels. These effects were still evident after a 14-day recovery period; however, the methemoglobin levels remained elevated in only 2/5 of the high-dose treated animals (Smith et al. 1988).

Following 10 sequential exposures, the methemoglobin levels were 0.2%, 0.87%, and 1.53% and then 0.2%, 0.13%, and 0.7% after a recovery period of 14 days. Further, erythrocyte numbers and the concentration of hemoglobin levels and the hematocrit decreased during exposure but had increased again after the 14-day recovery period (Smith et al. 1988). In

treated rats, the urine volume decreased in a dose-dependent manner during exposure and after a recovery period of 14 days. In high-dose 4-nitrophenol treated animals, the absolute spleen weight was significantly lower than the control group after the 10 sequential exposures, and the absolute or relative spleen and lung weights were significantly lower by comparison with controls after the 14-day recovery period. The authors of this study argued that the biological significance of the observed changes in organ weights was uncertain due in part to an absence of supporting evidence from pathological examinations (Smith et al. 1988).

In a complementary study, exposure to the dust of the sodium salt of 4-nitrophenol with a MMAD of 4.0–4.8 µm at doses of 0, 30, or 130 mg/m^3 also provoked irritation – although the mechanisms responsible for it remained unknown (Smith et al. 1988). Methemoglobinemia was evident in exposed animals; however, this was only observed at an exposure level of 130 mg/m^3 and the effect was reversible within a 14-day recovery period. Observed methemoglobin values were 0.5%, 0.3%, and 1.5% after 10 exposures and 0.4%, 0.5%, and 0.2% after 14 days of recovery. Gross and histopathological examination revealed no adverse effects in any of the dosed groups. Based on these observations, a derived NOAEL of 30 mg/m^3 was reported (Smith et al. 1988).

In a similar study, groups of Sprague-Dawley rats (n = 15 per gender) were exposed “whole body” to 0, 1, 5, or 30 mg/m^3 of 4-nitrophenol dust (with an MMAD of 5.2–6.7 µm) for 6 hours/day and 5 days/week, over a 4-week period (Hazleton Lab., 1983). On treatment with 4-nitrophenol dust, no deaths were reported and there were no changes in exposure-related effects that included hematological or clinical chemistry end-points, gross necroscopic and histopathological examination, and body or organ weights. In the high-dose 4-nitrophenol treated animals, it was noted that the animals displayed diffuse unilateral and bilateral anterior capsular cataracts.

The methemoglobin values that were determined after 2 weeks of exposure were highly variable and seemed unusually high (i.e., >3%) in some unexposed controls (Coate et al. 1983). The exposure group showed increased levels of methemoglobin following treatment with 5 mg/m^3 of 4-nitrophenol dust, which was a statistically significant increase in male rats but not in females (males: 0.8%, 0.5%, 2.2%, and 1.1%; females: 1.3%, 1.1%, 2.0%, and 1.0% respectively). Thus, based on local effects (i.e., development of cataracts), a NOAEL value of 5 mg/m^3 was derived. A NOAEL value for systemic effects (i.e., formation of methemoglobin) might be lower, but was not determined.

2.4.5 Mammalian Dermal Toxicity

2.4.5.1 Mammalian Dermal Toxicity—Acute

In the limit dermal toxicity test for 2-nitrophenol, the LD_{50} value for rats was calculated as >5,000 mg/kg body weight (Koerdel et al. 1981). The activity of 2-nitrophenol was found to be slightly irritating to the skin but not to the eye (although in the original articles, the scores for dermal toxicity were not given) (Koerdel et al. 1981). Further, in a Buehler test that was conducted with guinea-pigs, the substance did not display any skin-sensitizing effects (Koerdel et al. 1981).

Formation of methemoglobin was not detected after dermal application of a 50% solution of 2-nitrophenol in water to rabbits (BASF 1970 – as cited in Boehncke et al. 2000). However, in this study, although the dose was not specified, the exposure time for exposures on the back was 1 minute to 20 hours, and 20 hours for exposures that were done on the ear (BASF 1970 – as cited in Boehncke et al. 2000).

In one study that was performed according to the U.S. Food and Drug Administration (FDA) guidelines, non-dissolved 4-nitrophenol was found to be slightly irritating to the skin (i.e., scored 2 of 8 in a skin irritation assay; Hoechst 1977c). However, in another study that used methods comparable to those described in OECD Test Guideline 404, the non-dissolved 4-nitrophenol did not display any skin-irritating effects (i.e., scored 0 of 4; Andrae et al. 1981). In regard to ocular exposure, application of 4-nitrophenol as a 10% solution to the eyes was subsequently found to be slightly irritating in a test that was conducted according to the FDA guidelines (scores were not given in this study; Hoechst 1977c).

Table 4. Summary of Acute Dermal Toxicity for 4-Nitrophenol in Mammals

Test Organism	LD_{50} (mg/kg)	Test Results			Study
		NOAEL (mg/kg)	LOAEL (mg/kg)	Effects Observed at the LOAEL	
Rats (female)	1024	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Rats (female)	1275	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Rats (male)	1300	NA	NA	None stated	Eur. Com. 2000 (4-nitrophenol)
Rabbits (male)	3690	NA	NA	Dermal irritation at the application site	U.S. EPA 1998 (4-nitrophenol)

Legend:
Eur. Com. = European Commission
mg/kg = milligram per kilogram
mg/kg-day = milligram per kilogram per day
NOAEL= no observed adverse effect level
LOAEL = lowest observed adverse effect level
LD_{50} = dose expected to result in 50% lethality
NA = not applicable

Results that were derived from exposing animals to non-dissolved 4-nitrophenol in a test that was conducted according to FDA guidelines indicated that the substance was either strongly irritating (scores not given; Hoechst 1977c) or slightly irritating in a test that was comparable to OECD Test Guideline 405 (i.e., scored 1–2 of 4; Andrae et al. 1981). Additionally, in a guinea-pig maximization test that was comparable to OECD Test Guideline 406, skin sensitization was

shown in 5 of the 20 treated animals (Andrae et al. 1981). Data on respiratory tract sensitization for 2- and 4-nitrophenol were not identified from the current literature.

2.4.5.2 Mammalian Dermal Toxicity—Subchronic

There are presently few studies that have explored short- and long-term dermal exposures to mono-nitrophenols, and none are available for either 2- or 3-nitrophenol. From the available literature, subchronic and chronic studies of the dermal effects of mono-nitrophenols in animal models are only available for 4-nitrophenol.

In a subchronic dermal toxicity study, male and female Swiss-Webster mice (10 mice per gender and dose group) were exposed to 4-nitrophenol at doses of 0, 21.9, 43.8, 87.5, 175, or 350 mg/kg in acetone, which was applied to the interscapular skin 3 times per week for 13 weeks (U.S. EPA 1998). At a dose of 350 mg/kg, all male mice and eight female mice died before termination of the study. In addition, at 175 mg/kg, it was found that three male mice and one female mouse died following exposure. All deaths except one were attributed to treatment. Statistically significant increased incidence of epidermal inflammation (marked to severe), hyperplasia, and hyperkeratosis were observed in both male and female mice at 175 and 350 mg/kg. Dermal necrosis was observed in two to three males. Based on mortality and dermal irritation, a LOAEL of 175 mg/kg was derived from this study. The NOAEL of 87.5 mg/kg was similarly derived from observations based on mortality and dermal irritation. However, it was unknown from this study whether other parameters such as body weight, clinical chemistry and hematology, or clinical signs other than dermal lesions were evaluated (NTP 1993, U.S. EPA 1998).

2.4.5.3 Mammalian Dermal Toxicity—Chronic

2.4.5.3.1 Mammalian Dermal Toxicity—Chronic: Reproduction

A two-generation dermal reproduction study of 24 female and 12 male Sprague-Dawley rats, exposed animals to 4-nitrophenol that was dissolved in ethanol by dermal application at doses of 0, 50, 100, or 250 mg/kg body weight per day, for 5 days/week (Angerhofer 1985). The F_0 generation was exposed over a period of 140 days (20 weeks) before mating. Dosing of the F_0 females continued throughout breeding, gestation, and lactation. In addition, groups of 26 female and 13 male rats of the F_1 generation were exposed for 168 days (24 weeks) as described above for the F_0 rats. The female rats were again exposed throughout breeding, gestation, and lactation (Angerhofer 1985). There were dose-related signs of skin irritation (i.e., erythema, scaling, scabbing, and cracking) in dosed animals. However, gross and histopathological examination did not suggest any significant adverse effects after dermal treatment of the F_0 generation for 20 weeks and the F_1 generation for 24 weeks at a dose of up to 250 mg 4-nitrophenol/kg-day (Angerhofer 1985). All F_0 rats that received 4-nitrophenol as low as 50 mg/kg-day experienced a dose-related pattern of dermal irritation that consisted of varying degrees of skin lesions that included erythema, scaling, scabbing, and cracking.

Several rats also developed hyper-excitability. Moreover, offspring of 4-nitrophenol-dosed parents were unaffected in appearance, behavior and growth with no effects seen on body

weight. Dermal irritation in offspring (F_1 rats) was similar to that seen in F_0. The calculated indices concerning fertility, gestation, viability, and lactation were similar to those of controls. In addition, the testes-to-body weight ratios in the F_0 generation were unaffected by dermal exposure to 4-nitrophenol, and histological lesions were not observed in the testes. The reproductive NOAEL following dermal exposure was derived as ≥250 mg/kg. A LOAEL value was not established from this dataset (Angerhofer 1985).

2.4.5.3.2 Mammalian Dermal Toxicity – Chronic: Carcinogenicity

In a long-term carcinogenicity study in a Swiss-Webster mouse model (n = 50 per gender per dose group), the National Toxicology Program (NTP) explored dermatological effects by applying 4-nitrophenol in acetone to the interscapular skin at doses of 0, 40, 80, or 160 mg/kg, at a frequency of 3 days/week for 78 weeks (NTP 1993). It was concluded that there was no evidence of carcinogenic activity in male or female mice at any dose of applied 4-nitrophenol. At termination of the study, the survival rates were 29/60, 17/60, 26/60, and 24/60 for male mice and 35/60, 26/60, 33/60, and 27/60 for female mice. The increased mortality after 60 weeks was due to a generalized amyloidosis (the severity of the amyloidosis was similar among dosed and control animals) and secondary kidney failure. Thus, treatment did not necessarily affect animal survival. The final mean body weights of the dosed animals were similar to those of the controls.

No statistically significant or biologically noteworthy changes were observed in the incidence of 4-nitrophenol related neoplastic or non-neoplastic skin lesions at the site of application or the control site in male or female mice during this study. However, since observed increases in lung neoplasms were not dose-related and alveolar epithelial hyperplasia was not increased in any of the treatment groups, these neoplasms were likely unrelated to 4-nitrophenol application. Under the conditions of this 18-month dermal study, there was no evidence of carcinogenic activity in male or female mice that received 40, 80, or 160 mg/kg 4-nitrophenol (NTP 1993).

In another study, doses of 2-nitrophenol and 4-nitrophenol were applied twice a week for 12 weeks to 2–3 month old albino (Sutter) mice (Boutwell and Bosch 1959). No papilloma or epithelial skin tumors were observed in 31 female mice following application of a 20% solution of 2- or 4-nitrophenol in dioxane (Boutwell and Bosch 1959).

2.5 Mammalian Toxicity—Other

2.5.1 Mammalian Toxicity—Other: Endocrine Disruption

The results of uterotrophic and Hershberger assays in rats indicated that 4-nitrophenol (originally isolated from diesel exhaust particles) exhibited estrogen-like and anti-androgenic effects on female rats (Li et al. 2006). Daily subcutaneous injection of 4-nitrophenol in ovariectomized rats at doses of 1, 10, or 100 mg/kg for 7 days had no effect on body weight gain, nor did it significantly alter adrenal or pituitary gland weights. However, significantly decreased liver weights were seen following dosing with 0.1 and 1 mg/kg 4-nitrophenol, and kidney weight decreased significantly in the 0.1 mg/kg 4-nitrophenol group as compared with controls. In addition, in ovariectomized immature female rats that were injected subcutaneously

with 1, 10, or 100 mg/kg 4-nitrophenol daily for 7 days, significant increases in uterine weights were seen only in those rats that received 10 or 100 mg/kg ($P<0.05$).

Testosterone supplemented and castrated immature male rats that were subcutaneously injected with doses of 0.01, 0.1, or 1.0 mg/kg 4-nitrophenol for 5 days showed decreased seminal vesicles, ventral prostate, levator ani and bulbocavernosus muscles, and glans penis (Li et al. 2006). Subsequently, plasma FSH and LH levels remained unaltered in female rats but were significantly ($P<0.05$) increased in male rats on treatment with 0.1 mg/kg 4-nitrophenol.

The results indicated that 4-nitrophenol could promote both estrogenic and anti-androgenic activities *in vivo* (Li et al. 2006). In addition, by using a human androgen receptor (hAR)-yeast-screening assay it was shown that 4-nitrophenol possessed estrogenic activity in a colorimetric assay (Taneda et al. 2004). Anti-androgenic effects were demonstrated by a reduced response by hAR-yeast in the presence of 5-alpha-dihydrotesosterone (Taneda et al. 2004).

Figure 2 **4-NITROPHENOL: DERMAL HEALTH EFFECTS TO MAMMALS**

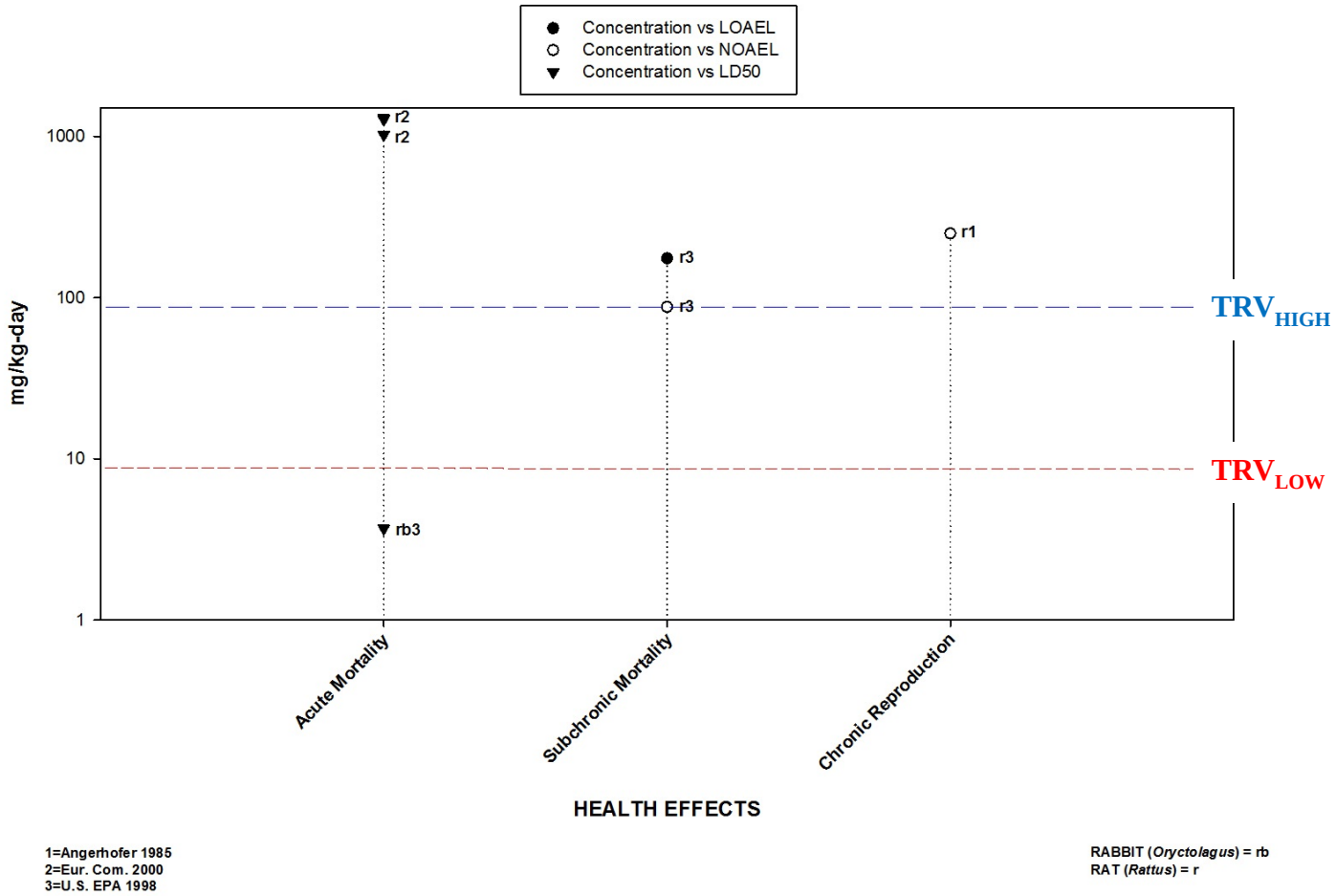


Figure 2. 4-Nitrophenol Dermal Health Effects to Mammals

Legend:
LOAEL = lowest observed adverse effect level
NOAEL = no observed adverse effect level
LD_{50}= dose expected to result in 50% lethality
Note: A UF of 10 was applied to the subchronic results to arrive at the TRVs shown here.

2.6 Summary of Avian Toxicology

2.6.1 Avian Acute Oral Toxicity

In studies submitted to the U.S. EPA (1998) for 4-nitrophenol, the oral LD_{50} in the Northern bobwhite (*Colinus virginianus*) was reported as 577 mg/kg with a NOAEL reported as 159 mg/kg (U.S. EPA 1979a). In acute dietary studies, LC_{50} values for both the Northern bobwhite and mallard (*Anas platyrhynchos*) were reported to be > 5,620 ppm (U.S. EPA 1979b and c).

2.6.2 Avian Chronic Oral Toxicity

In a rare long-term toxicological study, male Japanese quail (aged 4 weeks and weighing 80–90 g) were randomly divided into four groups (n = 25–26 per group) and dose-dependent responses to 4-nitrophenol were determined (Ahmed et al. 2015a). Avian treatment groups comprised the following groups:

- 1 (control group);
- 2 (4-nitrophenol at 0.01 mg/kg body weight – low-dose group);
- 3 (4-nitrophenol at 0.1 mg/kg body weight – mid-dose group); and
- 4 (4-nitrophenol at 1.0 mg/kg body weight – high-dose group).

The 4-nitrophenol crystals were dissolved in phosphate-buffered saline and orally gavaged to quail for 75 days (Ahmed et al. 2015a). Next, five quail per group were weighed and euthanized at days 45, 60, and 75 post-treatment for subsequent necropsy, histology and blood chemistry, and other cell or molecular biological studies including hepatocyte culture and quantitative polymerase chain reaction analyses. Following administration of 4-nitrophenol corticosterone levels were significantly increased at 45, 60, and 75 days at all doses. Low-dose 4-nitrophenol induced focal lymphocytic infiltrates in the liver. The mid-dose group showed hepatocyte degeneration with some necrosis and perilobular cirrhosis. The high-dose group showed multi areas of blood vessel congestion and focal lymphocytic infiltration with mild hepatocytes degeneration (Ahmed et al. 2015a).

In addition, at 60 days post-treatment, the expression of particular genetic biomarkers of metabolism and oxidative stress in the liver hepatocytes *in vitro* (e.g., CYP1A4, 1B1 enzyme, AhR1, and HO-1) were increased at 60 days post-treatment with some of these markers and CYP1A5 showing a decreased trend in expression by 75 days post-treatment *in vivo*. It was concluded that the functional integrity of the liver is disrupted by 4-nitrophenol exposure in Japanese quail.

In another study, Ahmed et al. (2015b) dosed male Japanese quails (28 days old) orally using a plastic stomach tube with 4-nitrophenol in saline at dose of 0, 0.01, 0.1, or 1.00 mg/g for 2.5 months. Testicular histopathology, hormones, caspase-3 and claudin-1 tight junction protein, as well as plasma hormones, were analyzed. The results showed that long-term exposure of 4-nitrophenol caused testicular changes, along with testicular and cloacal gland atrophy in all dose levels tested.

2.7 Summary of Amphibian Toxicology

No acute toxicological data for the effects of nitrophenol on amphibians was located; however, studies were identified that explored nitrophenol metabolism in frogs. Frank and Beyer (1986) administered ^{14}C-labeled 3-nitrophenol (4–6 mg/kg) to *Rana temporaria* and found that 85–93% of the administered dose was eliminated within 17 hours following dosing. The identified metabolites comprised glucuronide, sulfate, and trace levels of sulfites. George et al. (1987) studied excretion and metabolism of phenol, 4-nitrophenol, and 2-methylphenol in *Rana temporaria* and *Xenopus laevis*, and found that Xenopus excreted 90–95% of the dose and metabolized approximately 50–65% of the dosed concentration of 4-nitrophenol and 2-methyl-phenol within 24 hours, and to approximately the same extent for each of those two compounds. The excretion of phenols in both species were not significantly different. Xenopus is unable to form glucoronides metabolites, unlike Rana.

2.8 Summary of Reptilian Toxicology

No toxicological data for the effects of any of the nitrophenols on reptiles were identified from the available primary or secondary literature.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 Toxicity Reference Values for Mammals—Oral

Acute toxicity studies for ingestion of nitrophenols established LD_{50} values that ranged from 202–620 mg/kg in rats, and 380–626 mg/kg in mice for 4-nitrophenol (Table 2). The LD_{50} values were reported as 2,830 mg/kg in rats and 1,300 mg/kg in mice, and 930 mg/kg in rats for 2-nitrophenol. For ingestion of 3-nitrophenol, the reported LD_{50} values were 1,410 mg/kg in mice. Few clinical or pathological findings were reported; however, lethargy and dyspnea were reported in rats to doses as low as 70 mg/kg for 4-nitrophenol (Branch 1983). NOAELs were not reported for these data sets.

Acute and subchronic oral gavage studies with rats and mice ranged in duration from 8 days of dosing during gestation up to 13 weeks (Table 3). In many of the reports for mammalian species, mortality in adults that had been dosed with 4-nitrophenol was the only adverse effect reported. Koizumi et al. (2001) reported that dosing for 28 days caused deaths in 10 of 12 male or female rats, and that male rats exhibited a higher incidence of eosinophilic infiltrates in proximal tubular cells of the kidneys when dosed with 400 mg/kg. Acute mortality and wheezing, dyspnea, pallor, prostration, and languid behavior were reported in rats of both sexes at doses as low as 70 mg/kg when given for 13 weeks (Schulze 1989b). Female rats showed reduced gain in body weight after doses of 27.6 mg/kg on days 6–16 of gestation.

No oral exposure studies reporting chronic effects were found for review, and subchronic toxicity data were only available for 4-nitrophenol. The antecedent description of study results demonstrates that the best means for deriving a TRV for this compound was via subchronic toxicity data. Thus, Technical Guidance Document 254 (USACHPPM 2000) provides UFs of 10

for the NOAEL TRV, and no UF for the LOAEL TRV under conditions where the TRVs are based on a subchronic NOAEL. Use of these UFs and a NOAEL of 13.8 mg/kg-day gives a NOAEL TRV of 1.38 mg/kg-day and a LOAEL TRV of 13.8 mg/kg-day for 4-nitrophenol. Insufficient toxicity data were available to determine ingestion TRVs for 2-nitrophenol or 3-nitrophenol. For the above reasons, and due to the unavailability of quality dose-response data across an acceptable range of doses, bone mineral density analysis from these exposures was not possible.

Table 5. Selected Ingestion TRVs for 4-nitrophenol for the Class Mammalia

TRV	Dose	Confidence
TRV_{Low}	1.38 mg/kg-day[a]	Medium
TRV_{High}	13.8 mg/kg-day[a]	Medium

Legend:
NOAEL = no observed adverse effect level
LOAEL = lowest observed adverse effect level

Note:
[a]U.S. EPA 1998

3.1.2 Toxicity Reference Values for Mammalia—Inhalation

A single acute toxicity study for inhalation of 4-nitrophenol reported no mortality in rats that were exposed to up to 4.7 mg/L with the only clinical signs being transient weight loss and corneal opacity (Smith et al. 1988).

Subchronic inhalation studies in rats ranged in duration from 10 days of dosing up to 4 weeks. No mortality was reported at exposure concentrations as high as 2.5 mg/L. Exposure to 0.01 mg/L caused methemoglobinemia, and exposure to 2.5 mg/L caused elevated numbers of erythrocytes and concentrations of hemoglobin. In addition, increased hematocrit levels, SGOT, and creatinine were observed, as well as lower absolute and relative spleen and lung weights (Smith et al. 1988). No chronic inhalation studies were available.

No studies of chronic exposure were available. Since elevated methemoglobin was noted in a single subchronic test without having established a NOAEL, there is insufficient data to develop an inhalation TRV for 4-nitrophenol.

3.1.3 Toxicity Reference Values for Mammals—Dermal

The acute dermal LD_{50} in male rabbits was reported for 4-nitrophenol and determined to be 3.69 mg/kg (U.S. EPA 1998), and 1,024–1,275 mg/kg in female rats or 1,300 mg/kg in male rats (Eur. Com. 2000). The only adverse effect reported was dermal irritation at the application site (U.S. EPA 1998). In one subchronic study, the NOAEL in mice for mortality was 87.5 mg/kg,

and the NOAEL for epidermal inflammation (marked severe), hyperplasia, and hyperkeratosis was also 87.5 mg/kg.

In a dermal reproduction study with rats, no reproductive effects occurred and the only adverse effects were dose-related dermal irritation consisting of varying degrees of erythema, scaling, scabbing, and cracking. The NOAEL for reproduction, 250 mg/kg, was the highest dose tested.

The LOAEL for dermal effects was 50 mg/kg-day, which was the lowest dose tested (Angerhofer 1985). A chronic carcinogenicity test with mice showed no carcinogenic response or chemical-related non-neoplastic skin lesions at the application site in either male or female mice at doses up to 160 mg/kg. From this data, the derived NOAEL was determined to be 160 mg/kg (NTP, 1993). Neither of the available chronic studies established reliable LOAELs; however, adverse effects were noted in a subchronic test with a NOAEL of 87.5 mg/kg that was reported for epidermal inflammation. Therefore, the aforementioned subchronic toxicity results were used to develop the TRVs. The TRV Protocol (USACHPPM 2000) provides an uncertainty factor of 10 for the NOAEL TRV and no uncertainty factor for the LOAEL TRV when the TRVs are based on a subchronic NOAEL. Use of these uncertainty factors and the NOAEL of 87.5 mg/kg results in a NOAEL TRV of 8.75 mg/kg and a LOAEL TRV of 87.5 mg/kg. No TRVs are available for 2-nitrophenol or 3-nitrophenol.

Table 6. Selected Dermal TRVs of 4-nitrophenol for the Class Mammalia

TRV	Dose	Confidence
TRV_{Low}	8.75 mg/kg-day[a]	Low
TRV_{High}	87.5 mg-kg-day[a]	Low

Legend:
NOAEL = no observed adverse effect level

Note:
[a]U.S. EPA 1998

3.2 Toxicity Reference Value for Birds

A single acute avian toxicity study reported an LD_{50} of 577 mg/kg for 4-nitrophenol with a NOAEL of 159 mg/kg in Northern Bobwhite quail (U.S. EPA 1979a). Additional acute studies reported a dietary LC_{50} from a 5-day feeding study that exceeded 5,260 ppm for young Quail and Mallard ducks (U.S. EPA 1998). Since these studies do not relate the body weights of growing, juvenile birds to the body weights of adult birds based on dietary exposures, these studies do not provide sufficient evidence from which to derive TRVs. Only one chronic toxicity study (Ahmed et al. 2015a) was available for birds. However, data from that report was largely descriptive, mechanistic, or observational. The study lacked quality data to enable determination of an LD_{50} or derivation of NOAEL or LOAEL values.

Since only one acute toxicity study was available to provide information on toxicological effects in birds, the acute results were used to derive a TRV approximation. TG254 (USACHPPM 2000) provides an uncertainty factor of 30 for the NOAEL TRV, and no uncertainty factor for the LOAEL TRV, since the TRVs are approximated from an acute NOAEL. Use of an uncertainty factor of 30 and the NOAEL of 159 mg/kg provides a NOAEL TRV of 5.3 mg/kg and a LOAEL TRV of 159 mg/kg. No TRVs are available for 2-nitrophenol or 3-nitrophenol.

Table 7. Selected Ingestion TRVs for Class Aves

TRV	Dose	Confidence
TRV_{Low}	5.3 mg/kg[a]	Low
TRV_{High}	159 mg/kg[a]	Low

Legend:
NOAEL = no observed adverse effect level
LOAEL = lowest observed adverse effect level

Note:
[a]U.S. EPA, 1979a

3.3 Toxicity Reference Values for Amphibians

Not available at this time.

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

3.5 Important Research Needs

Lack of data on the wildlife toxicity of mono-nitrophenols (particularly 3-nitrophenol—with the bulk of the studies focusing on 4-nitrophenol and, to a lesser extent, on 2-nitrophenol—weakens confidence in the development of a comprehensive TRV. Hence, additional toxicological studies of each of the mono-nitrophenol isomers are recommended. The majority of the acute toxicity studies across taxa that were reviewed in this report failed to report NOAELs.

More studies with multiple doses are required to establish a sufficient dose-response curve and point of departure estimation. Most acute studies did not include sufficiently high doses of exposure to compounds to cause mortality. Thus, the doses at which mortality would occur are poorly defined. No oral chronic toxicity studies were available. This means that the lower limits of exposure that would lead to chronic adverse effects are not understood. Inhalation and dermal toxicity testing is limited for laboratory mammals and completely lacking for all other groups. No toxicity data are available for amphibians and reptiles, and the limited avian studies are restricted to acute exposures and one observational chronic toxicity study (Ahmed et al. 2015b).

Studies that have focused on both acute and chronic toxicity against wildlife mammals and non-mammalian wildlife including birds, reptiles, and amphibians are particularly warranted to enable future TRV development. Further studies are also needed to determine whether endocrine-disrupting effects of the mono-nitrophenol isomers occur in taxa other than mammals, and to accurately determine the doses at which they are active and relevant for TRV development.

APPENDIX A

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APPENDIX B

LITERATURE REVIEW

A very broad search on October 9, 2013 using DTIC's advance search function used the single search term, nitrophenol. This search identified 728 documents.

Additional focused searches on October 9–30, 2013 using DTIC's Multisearch function used the terms:

nitrophenol + quail. This search identified 20 documents.

nitrophenol + mallard. This search identified 22 documents.

nitrophenol + bird. This search identified 101 documents.

nitrophenol + avian. This search identified 34 documents.

nitrophenol +mouse. This search identified 172 documents.

nitrophenol +mice. This search identified 205 documents.

nitrophenol +rat. This search identified 358 documents.

nitrophenol +mammal. This search identified 133 documents.

nitrophenol + wildlife. This search identified 125 documents.

nitrophenol + ecotox*. This search identified 8 documents.

nitrophenol + amphib*. This search identified 0 documents.

nitrophenol + frog. This search identified 49 documents.

nitrophenol + *Xenopus*. This search identified 12 documents.

nitrophenol + reptil*. This search identified 35 documents.

On October 30, 2013, a search of the U.S. EPA's online Ecotox® database used the CAS No. 25154-55-6. No reference for amphibians, reptiles, birds or mammals were identified.

A search of the TOXLINE® database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on October 30, 2013 used the CAS No. 25154-55-6 as the search term. A total of 4600 articles were identified. This search was refined with:

25154-55-6 AND ecotox* resulted in 75 hits

25154-55-6 AND reptil* resulted in 2 hit

25154-55-6 AND amphib* resulted in 3 hits

25154-55-6 AND *Xenopus* resulted in 2 hits

25154-55-6 AND frog resulted in 7 hits

25154-55-6 AND salamander resulted in 0 hits

25154-55-6 AND avian resulted in 6 hits

25154-55-6 AND mallard resulted in 0 hits

25154-55-6 AND quail resulted in 12 hits

25154-55-6 AND bird* resulted in 21 hits

25154-55-6 AND wildlife resulted in 14 hits

25154-55-6 AND mammal* resulted in 227 hits

25154-55-6 AND rat resulted in 824 hits

25154-55-6 AND mouse resulted in 558 hits

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on October 30, 2013 used the CAS No. 100-02-7 as the search term. A total of 1324 articles were identified. This search was refined with:

100-02-7 AND ecotox* resulted in 35 hits

100-02-7 AND reptil* resulted in 1 hit

100-02-7 AND amphib* resulted in 0 hits

100-02-7 AND *Xenopus* resulted in 1 hits

100-02-7 AND frog resulted in 1 hits

100-02-7 AND salamander resulted in 0 hits

100-02-7 AND avian resulted in 1 hits

100-02-7 AND mallard resulted in 0 hits

100-02-7 AND quail resulted in 0 hits

100-02-7 AND bird* resulted in 4 hits

100-02-7 AND wildlife resulted in 5 hits

100-02-7 AND mammal* resulted in 69 hits

100-02-7 AND rat resulted in 130 hits

100-02-7 AND mouse resulted in 57 hits

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on November 19, 2013 used the CAS No. 554-84-7 as the search term. A total of 215 articles were identified. This search was refined with:

554-84-7 AND ecotox* resulted in 10 hits

554-84-7 AND reptil* resulted in 0 hit

554-84-7 AND amphib* resulted in 0 hits

554-84-7 AND *Xenopus* resulted in 0 hits

554-84-7 AND frog resulted in 2 hits

554-84-7 AND salamander resulted in 0 hits

554-84-7 AND avian resulted in 0 hits

554-84-7 AND mallard resulted in 0 hits

554-84-7 AND quail resulted in 0 hits

554-84-7 AND bird* resulted in 0 hits

554-84-7 AND wildlife resulted in 0 hits

554-84-7 AND mammal* resulted in 1 hits

554-84-7 AND rat resulted in 10 hits

554-84-7 AND mouse resulted in 5 hits

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on November 20, 2013 used the CAS No. 88-75-5 as the search term. A total of 530 articles were identified. This search was refined with:

88-75-5 AND ecotox* resulted in 22 hits

88-75-5 AND reptil* resulted in 0 hits

88-75-5 AND amphib* resulted in 0 hits

88-75-5 AND *Xenopus* resulted in 0 hits

88-75-5 AND frog resulted in 0 hits

88-75-5 AND salamander resulted in 0 hits

88-75-5 AND avian resulted in 0 hits

88-75-5 AND mallard resulted in 1 hits

88-75-5 AND quail resulted in 0 hits

88-75-5 AND bird* resulted in 3 hits

88-75-5 AND wildlife resulted in 0 hits

88-75-5 AND mammal* resulted in 3 hits

88-75-5 AND rat resulted in 20 hits

88-75-5 AND mouse resulted in 5 hits

Searches of the BIOSIS database, on November 21, 2013, used a number of keyword combinations to capture articles that might have been missed in the broader searches. These combinations were:

25154-55-6 AND ecotox* resulted in 0 hits

25154-55-6 AND reptil* resulted in 1 hit

25154-55-6 AND amphib* resulted in 0 hits
25154-55-6 AND *Xenopus* resulted in 0 hits
25154-55-6 AND frog resulted in 0 hits
25154-55-6 AND salamander resulted in 0 hits
25154-55-6 AND avian resulted in 0 hits
25154-55-6 AND mallard resulted in 0 hits
25154-55-6 AND quail resulted in 0 hits
25154-55-6 AND bird* resulted in 3 hits
25154-55-6 AND wildlife resulted in 1 hit
25154-55-6 AND mammal* resulted in 77 hits
25154-55-6 AND rat resulted in 25 hits
25154-55-6 AND mouse resulted in 13 hits

100-02-7 AND ecotox* resulted in 14 hits
100-02-7 AND reptil* resulted in 5 hit
100-02-7 AND amphib* resulted in 7 hits
100-02-7 AND *Xenopus* resulted in 3 hits
100-02-7 AND frog resulted in 4 hits
100-02-7 AND salamander resulted in 0 hits
100-02-7 AND avian resulted in 4 hits
100-02-7 AND mallard resulted in 0 hits
100-02-7 AND quail resulted in 3 hits
100-02-7 AND bird* resulted in 30 hits
100-02-7 AND wildlife resulted in 7 hits
100-02-7 AND mammal* resulted in 346 hits
100-02-7 AND rat resulted in 173 hits
100-02-7 AND mouse resulted in 102 hits

554-84-7 AND ecotox* resulted in 1 hits
554-84-7 AND reptil* resulted in 0 hit
554-84-7 AND amphib* resulted in 5 hits

554-84-7 AND *Xenopus* resulted in 0 hits

554-84-7 AND frog resulted in 5 hits

554-84-7 AND salamander resulted in 0 hits

554-84-7 AND avian resulted in 0 hits

554-84-7 AND mallard resulted in 0 hits

554-84-7 AND quail resulted in 0 hits

554-84-7 AND bird* resulted in 0 hits

554-84-7 AND wildlife resulted in 2 hits

554-84-7 AND mammal* resulted in 15 hits

554-84-7 AND rat resulted in 5 hits

554-84-7 AND mouse resulted in 4 hits

88-75-5 AND ecotox* resulted in 1 hit

88-75-5 AND reptil* resulted in 0 hits

88-75-5 AND amphib* resulted in 0 hits

88-75-5 AND *Xenopus* resulted in 0 hits

88-75-5 AND frog resulted in 0 hits

88-75-5 AND salamander resulted in 0 hits

88-75-5 AND avian resulted in 0 hits

88-75-5 AND mallard resulted in 0 hits

88-75-5 AND quail resulted in 0 hits

88-75-5 AND bird* resulted in 0 hits

88-75-5 AND wildlife resulted in 1 hits

88-75-5 AND mammal* resulted in 37 hits

88-75-5 AND rat resulted in 7 hits

88-75-5 AND mouse resulted in 5 hits

The different searches defined above identified many of the same articles. Additional references were identified during the review of individual articles. A total of 106 articles were reviewed.

In addition, during the revision and updating of the report, On 2 January 2018, the original draft was updated and an additional literature search was conducted using the Johns Hopkins Welch Medical Library Multisearch Database.

Using nitrophenol as a single search term in the title of the document, this search strategy identified 3,284 documents in Web of Science; 1,073 documents in PubMed; 0 documents in CINAHL Plus; 1,073 documents in MEDLINE®; 0 documents in WorldCat; and 1,520 documents in Academic Search Complete.

For comprehensive targeted searches, the above specific databases and others (as indicated below) were searched with the aim of refining the identification of specific articles of potential interest.

A standard Boolean operator search of PubMed® (National Library of Medicine, NIH) of nitrophenol [TI] as the anchored word in the title with the following search strings were selected using wild-card (*) for optimal returns on search terms and contexts:

nitrophenol [TI] AND Tox* returned 188 hits

nitrophenol [TI] AND Tox* refined to 2018 returned 4 hits

nitrophenol [TI] AND Tox* refined to 2017 returned 20 hits

nitrophenol [TI] AND Tox* refined to 2016 returned 21 hits

nitrophenol [TI] AND Tox* refined to 2015 returned 13 hits

nitrophenol [TI] AND Tox* refined to 2014 returned 6 hits

Species-specific search strings yielded the following hits from PubMed:

nitrophenol [TI] AND mammal returned 299 hits

nitrophenol [TI] AND animal returned 282 hits

nitrophenol [TI] AND quail returned 7 hits

nitrophenol [TI] AND mallard returned 0 hits

nitrophenol [TI] AND bird returned 13 hits

nitrophenol [TI] AND avian returned 13 hits

nitrophenol [TI] AND mouse returned 37 hits

nitrophenol [TI] AND mice returned 37 hits

nitrophenol [TI] AND rat returned 154 hits

nitrophenol [TI] AND wildlife returned 6 hits

nitrophenol [TI] AND ecotox* returned 11 hits

nitrophenol [TI] AND amphib* returned 0 hits

nitrophenol [TI] AND amphibian returned 3 hits

nitrophenol [TI] AND frog returned 0 hits

nitrophenol [TI] AND *Xenopus* returned 1 hit

nitrophenol [TI] AND reptile returned 1 hit

nitrophenol [TI] AND reptil* returned 0 hits

A repeat search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on 2 January 2018, used the CAS No. 25154-55-6 as the search term. A total of 4,600 articles were identified. This search was refined with:

25154-55-6 AND ecotox* resulted in 90 hits

25154-55-6 AND reptil* resulted in 2 hits

25154-55-6 AND amphib* resulted in 3 hits

25154-55-6 AND *Xenopus* resulted in 3 hits

25154-55-6 AND frog resulted in 9 hits

25154-55-6 AND salamander resulted in 0 hits

25154-55-6 AND avian resulted in 4 hits

25154-55-6 AND mallard resulted in 0 hits

25154-55-6 AND quail resulted in 13 hits

25154-55-6 AND bird* resulted in 14 hits

25154-55-6 AND wildlife resulted in 29 hits

25154-55-6 AND mammal* resulted in 133 hits

25154-55-6 AND rat resulted in 1,144 hits

25154-55-6 AND mouse resulted in 468 hits

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on 2 January 2018 used the CAS No. 100-02-7 as the search term. A total of 1,324 articles were identified. This search was refined with:

100-02-7 AND ecotox* resulted in 88 hits

100-02-7 AND reptil* resulted in 2 hits

100-02-7 AND amphib* resulted in 2 hits

100-02-7 AND *Xenopus* resulted in 2 hits

100-02-7 AND frog resulted in 4 hits

100-02-7 AND salamander resulted in 0 hits

100-02-7 AND avian resulted in 4 hits

100-02-7 AND mallard resulted in 0 hits

100-02-7 AND quail resulted in 12 hits

100-02-7 AND bird* resulted in 14 hits

100-02-7 AND wildlife resulted in 27 hits

100-02-7 AND mammal* resulted in 69 hits

100-02-7 AND rat resulted in 901 hits

100-02-7 AND mouse resulted in 282 hits

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on 19 November 2013, used the CAS No. 554-84-7 as the search term. A total of 215 articles were identified. This search was refined with:

554-84-7 AND ecotox* resulted in 12 hits

554-84-7 AND reptil* resulted in 0 hits

554-84-7 AND amphib* resulted in 0 hits

554-84-7 AND *Xenopus* resulted in 0 hits

554-84-7 AND frog resulted in 3 hits

554-84-7 AND salamander resulted in 0 hits

554-84-7 AND avian resulted in 0 hits

554-84-7 AND mallard resulted in 0 hits

554-84-7 AND quail resulted in 0 hits

554-84-7 AND bird* resulted in 0 hits

554-84-7 AND wildlife resulted in 0 hits

554-84-7 AND mammal* resulted in 1 hit

554-84-7 AND rat resulted in 12 hits

554-84-7 AND mouse resulted in 6 hits

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on 20 November 2013, used the CAS No. 88-75-5 as the search term. A total of 530 articles were identified. This search was refined with:

88-75-5 AND ecotox* resulted in 27 hits

88-75-5 AND reptil* resulted in 0 hits

88-75-5 AND amphib* resulted in 0 hits

88-75-5 AND *Xenopus* resulted in 0 hits

88-75-5 AND frog resulted in 0 hits

88-75-5 AND salamander resulted in 0 hits

88-75-5 AND avian resulted in 0 hits

88-75-5 AND mallard resulted in 1 hits

88-75-5 AND quail resulted in 0 hits

88-75-5 AND bird* resulted in 3 hits

88-75-5 AND wildlife resulted in 0 hits

88-75-5 AND mammal* resulted in 4 hits

88-75-5 AND rat resulted in 42 hits

88-75-5 AND mouse resulted in 24 hits

All searched and returned hits for each of the CAS numbers and search terms were interrogated for specific criteria that included relevance, quality and content and included in this WTA report had they displayed those criteria.

During the revision of this WTA, the above literature search strategies were repeated through June 2019 for additional articles that could assist in the development of WTA TRVs. No additional articles deemed relevant or of the required quality were identified during those searches.