

201-15339A

HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For the

CHLORONITROBENZENE CATEGORY

CAS Number 88-73-3; Benzene, 1-Chloro-2-Nitro-

CAS Number 121-73-3; Benzene, 1-Chloro-3-Nitro-

CAS Number 100-00-5; Benzene, 1-Chloro-4-Nitro-

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EXECUTIVE SUMMARY

Solutia Inc. voluntarily submits the following Category Justification, Screening Information Data (Robust Summaries) and Test Plan for review under the Environmental Protection Agency's High Production Volume (HPV) Chemicals Challenge Program. The category, entitled "Chloronitrobenzenes" consists of three members, Benzene, 1-chloro-2-nitro-, also known as o-Chloronitrobenzene (CAS No. 88-73-3), Benzene, 1-chloro-3-nitro-, also known as m-Chloronitrobenzene (CAS No. 121-73-3), and Benzene, 1-chloro-4-nitro-, also known as p-Chloronitrobenzene (CAS No. 100-00-5). This category is justified on the basis of chemical structure similarity, as well as similarity of basic screening data, as provided in an initial assessment of physico-chemical properties, environmental fate and human and environmental effects.

A substantial amount of data exists to evaluate the potential hazards associated with this Category of chemicals. Use of key studies available from data already developed or derived from recommended estimation models provide adequate support to characterize each Endpoint in the HPV Chemicals Challenge Program without the need for additional testing.

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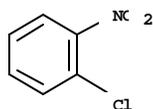
TEST PLAN FOR CHLORONITROBENZENES

I. INTRODUCTION AND IDENTIFICATION OF CATEGORY MEMBERS

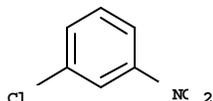
Under EPA's High Production Volume (HPV) Chemicals Challenge Program, Solutia Inc. has committed to voluntarily compile basic screening data on three chemicals of similar structure, namely Benzene, 1-chloro-2-nitro (known as o-chloronitrobenzene or ONCB; CAS no. 88-73-3), Benzene, 1-chloro-3-nitro (known as m-chloronitrobenzene or MNCB; CAS no. 121-73-3) and Benzene, 1-chloro-4-nitro (known as p-chloronitrobenzene or PNCB; CAS no. 100-00-5). Solutia Inc. believes that a category of Chloronitrobenzenes is scientifically justifiable. The data included in this Category involve physicochemical properties, environmental fate, and human and environmental effects of the chemicals in this Category, as defined by the Organization for Economic Cooperation and Development (OECD). Most of the information provided comes from existing data developed on behalf of Solutia Inc., or its predecessor Monsanto Co., much of which has already been submitted to the US EPA under auspices of sections of the Toxic Substances Control Act and is available through TSCATS; additional information can be found in the published scientific literature or from recommended estimation models. This submission fulfills Solutia's obligation to the HPV Challenge Program for these three chemicals.

A. Structure and Nomenclature

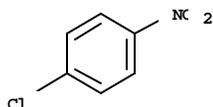
The members of this family of Chloronitrobenzenes, include the following chemicals:



- a. Benzene, 1-chloro-2-nitro-
CAS No. 88-73-3
Synonyms: o-Nitrochlorobenzene; o-Chloronitrobenzene; ONCB



- b. Benzene, 1-chloro-3-nitro-
 CAS No. 121-73-3
 Synonyms: m-Nitrochlorobenzene; m-Chloronitrobenzene; MNCB;



- c. Benzene, 1-chloro-4-nitro-
 CAS No. 100-00-5
 Synonyms: p-Nitrochlorobenzene; p-Chloronitrobenzene; PNCB;

B. Manufacturing & Use

Members of the Chloronitrobenzenes Category, p-nitrochlorobenzene (PNCB), o-nitrochlorobenzene (ONCB) and m-nitrochlorobenzene (MNCB), are manufactured by a single US producer, Solutia Inc., at a single manufacturing site in an essentially closed, continuous process. Only a few employees are involved in the manufacturing operations and have minimal potential for skin or airborne exposure, which occurs chiefly during material transfer operations.

All three Chloronitrobenzene isomers, PNCB, ONCB and MNCB are known to produce methemoglobinemia in human and animals (Linch, 1974) and are considered hazardous after dermal contact. Addition of the nitro group in the *para* position relative to the chlorine group on the benzene molecule results in the formation of the most toxic of the three isomers. Potency of response in both humans and animals is equivalent to *para*>*meta*>>*ortho* (Watanabe et al, 1976; Davydova, 1967). To minimize the potential for adverse health effects due to methemoglobinemia resulting from occupational exposure via inhalation or skin absorption, a TLV ® of 0.1 ppm (~0.64 mg/m³) has been established for PNCB (ACGIH, 2001). While comparative toxicity and occupational experience indicate that MNCB and ONCB produce less toxicity and a lower risk of methemoglobinemia, an internal Solutia Inc. occupational standard of 1 mg/m³ has also been set for these chemicals. In all cases, specific manufacturing procedures and practices have been established to minimize occupational exposure potential.

PNCB and ONCB are important chemical intermediates that serve as basic building blocks for the manufacture of numerous industrial chemicals. For example, PNCB is utilized via chemical reaction to make industrial chemicals that are ultimately used in the preparation of dyes and pigments, pesticides, and animal feed ingredients. ONCB is converted in similar fashion to dyes and pigments, polymer additives, veterinary pharmaceuticals and water-treatment chemicals. MNCB has limited use as a chemical intermediate.

Chloronitrobenzenes are sold to a limited number of customers at a few processing sites for the express purpose of full chemical conversion into other industrial chemicals. There are no known or suspected consumer exposures to these chemicals resulting from TSCA-related activities, as they are fully consumed as chemical intermediates.

II. CATEGORY JUSTIFICATION

For purposes of the HPV Challenge Program, EPA has provided guidance as to the definition and justifications to be used in selection of a chemical Category (US EPA, 1999c). The definition states that a chemical Category should be “a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity”. Solutia Inc. has opted to form the Chloronitrobenzene Category with this guidance in mind.

Common Structure

The three chemicals selected for inclusion in this category are isomeric forms of the same base chemical, nitrobenzene. Hence, they are of common structure.

Common Functional Groups

Each of these nitrobenzene compounds are aromatic hydrocarbons for which one benzene ring hydrogen has been replaced by a nitro (NO₂) radical and one benzene ring hydrogen further replaced with a chloro (Cl) group; the position (either *ortho* to, *meta* to, or *para* to the chloro grouping) of the ring placement of the nitro grouping is the only structural difference between these three isomers. For the most part, these compounds are similar in chemical properties, as well as in their pharmacological or toxicological effects. As such these effects are modified to a greater or lesser degree by the location of the substituent radicals (Beard and Noe, 1982; Davydova, 1967; Watanabe et al, 1976).

Similar or even Identical Properties or Hazards

Physicochemical properties of these three isomeric forms of the same chemical are quite similar. Their physical form is crystalline and their molecular weights and specific gravity are identical. Other parameters are similar, but not identical. A summary of available physicochemical data can be found in Table 4.

Environmental Fate data are summarized in Table 5. A large body of published information exists in this data category. Whether measured or estimated, there appears close agreement in each of the HPV Endpoints recorded for each of the chemicals in this category.

Comparative aquatic toxicity of the members of this Category can be found in Table 6. As shown, a similar degree of toxicity has been observed across the multiple test species included in this dataset.

Tables 7 - 10 summarize the comparative mammalian toxicity of these chemicals. It is well recognized that all three of these chemicals possess a similar mode of action. Their toxicity is characterized by a common and outstanding property, i.e., their ability to form methemoglobin (Beard and Noe, 1982) in both humans and animals. Comparative investigations have established the order of potency to be: para isomer > meta isomer >> ortho isomer (Watanabe et al, 1976; Davydova, 1967). However, there are marked species differences in susceptibility to methemoglobinemia with humans being decidedly more affected than rodent species. Thus, results of acute toxicity studies in rodents are not considered fully representative of the high acute toxicity to humans that can be elicited by these chemicals. On the basis of past human experience, where dermal contact or inhalation exposures resulted in incidences of methemoglobinemia, unusually diligent care has been taken to insure proper handling of both chemicals (each treated equally) during manufacture, shipment, disposal and use.

Thus, similarities in the chemical structure, biological mode of action and the extensive comparative data sets presented support use of a Category approach for these chemicals.

III. TEST PLAN RATIONALE

The information obtained and included to support this Test Plan have come from either 1) internal studies conducted by/or for Solutia Inc. (or its predecessor Monsanto Co.), 2) have been extracted from the scientific literature either as primary references or as found in well-accepted, peer-reviewed reference books, or 3) were estimated using environmental models accepted by the US EPA (1999b) for such purposes. This initial assessment includes information on physicochemical properties, environmental fate, and human and environmental effects associated with each member of this Category. The data used to support this program include those endpoints identified by the US EPA (1998); key studies have been identified for each data Endpoint and summarized in Robust Summary form and included in Section VII of this dossier.

All studies were reviewed and assessed for reliability according to standards specified by Klimisch *et al* (1997), as recommended by the US EPA (1999a). The following criteria were used for codification:

1. Reliable without Restriction - Includes studies which comply with US EPA and/or OECD-accepted testing guidelines, which were conducted using Good Laboratory Practices (GLPs) and for which test parameters are complete and well documented,
2. Reliable with Restriction – Includes studies which were conducted according to national/international testing guidance and are well documented. May include studies conducted prior to establishment of testing standards or GLPs but meet the test parameters and data documentation of subsequent guidance; also includes studies with test parameters which are well documented and scientifically valid but vary slightly from current testing guidance. Also included were physical-chemical property data obtained from reference handbooks as well as environmental endpoint values obtained from an accepted method of estimation (i.e. EPIWIN).
3. Not Reliable – Includes studies in which there are interferences in either the study design or results that provide scientific uncertainty or where documentation is insufficient.
4. Not Assignable – This designation is used in this dossier for studies which appear scientifically valid but for which insufficient information is available to adequately judge robustness.

Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs in this Dossier. Those key studies selected for inclusion are considered typical of the Endpoint responses observed in other studies of a similar nature and design, which were identified during our search of the literature; additional references can be found in the current ECB IUCLID dossiers for o-Chloronitrobenzene (2000), m-Chloronitrobenzene (2000) and p-Chloronitrobenzene (2000), as referenced below.

IV. TEST PLAN SUMMARIES AND CONCLUSIONS

The referenced available data for each Category member have been placed in an Endpoint-specific matrix and summarized individually in Table 1 (ONCB), Table 2 (MNCB) and Table 3 (PNCB). Substantial data exists for each chemical to evaluate its potential hazards in this screening level assessment. Where an HPV Endpoint has been untested, the need for testing has been assessed (1) with the understanding that these chemicals behave in a similar and/or predictable manner, and (2) by interpolation (i.e. Read-Across technique) between data from other key studies already available. Thus, we have used preexisting data, where possible, to support our assessment of potential hazards of the chemicals in this Category and avoid the unnecessary testing of additional laboratory animals.

Conclusion: All HPV Endpoints have been satisfied for the three Chloronitrobenzene isomers with data from studies that were either well documented, used OECD guideline methods and conducted in accord with GLPs, or were estimated from acceptable estimation modeling programs. Use of the “Read Across” technique was employed sparingly to support a limited number of endpoints. Hence, no further testing for any of the HPV endpoints is deemed necessary (Tables 1, 2 and 3).

Physical-chemical property values - Melting Point and Boiling Point values for all three Chloronitrobenzenes were obtained from reputable references and cited as an Accepted or Peer Reviewed value in their respective Hazardous Substances Data Banks (2002). Measured values were found for Vapor Pressures and Partition Coefficients from reputable studies, and which were also cited in accepted peer reviewed documents. Experimental values for the water solubility of each isomer have been published and are supported by an accepted estimation method. These values were given a classification of “2-Reliable with restrictions”.

Environmental Fate values describing Transport (Fugacity) for ONCB, MNCB and PNCB were obtained using a computer estimation –modeling program (EPIWIN, 2002) recommended by EPA and classified as “2-Reliable with restrictions”.

Photodegradation (direct) and Biodegradation data for each of the three Chloronitrobenzene isomers were characterized in well-documented studies, the latter conducted according to ASTM/EPA guidelines that since have been codified and are similar to OECD test #301 guidance. These studies thus are classified as “2-Reliable with restrictions” Indirect photodegradation was estimated with EPIWIN and separate robust summaries for indirect photodegradation are included. . No specific data were found for water stability of the chloronitrobenzenes. The hydrolysis of chloronitrobenzenes to chlorophenols (hydrolysis of the nitro group) or to nitrophenols (hydrolysis if the chloro group) are both thermodynamically feasible, as the enthalpy of reaction calculated from bond energies indicates hydrolysis to be a thermodynamically favored process (see robust summaries). The free energy of the transition state for this hydrolysis; however, is so high that the reactions are generally not feasible (March’s Advanced Organic Chemistry, fifth ed 2001 page 433). Special aromatic compounds, such as those with multiple electron withdrawing groups ortho and para to the halogen are potentially activated; however, even picryl chloride (with three nitro groups ortho or para to the chlorine) is stable in water at room temperature as it is transported with about 10% water to limit explosion potential. Thus, ortho and para CNB are anticipated to be hydrolysable under extreme conditions but are considered to have a hydrolytic half-life greater than one year under environmental conditions and the meta isomer is expected to be even more stable to hydrolysis. As chemical principles are considered reliable methodology, the reliability score is assigned as “2”.

Ecotoxicity – Acute Fish, Invertebrate and Plant Toxicity Endpoints for PNCB and ONCB have been fulfilled with studies, most of which were conducted according to US EPA test guidance consistent with OECD test guidelines. All studies were well documented and were designated “2-Reliable with restrictions”. An Acute Fish Toxicity study, also designated as “2-Reliable with restrictions”, has been included for MNCB. The Acute Invertebrate and Plant Toxicity Endpoints for MNCB are fulfilled using the ‘Read Across’ method of data evaluation, as no fully reliable studies were found in these two areas. Utility of this methodology is strengthened by comparative use of estimation modeling data as well as literature information deemed limited (“4-Not Assignable”) in documentation, but useful for supportive purposes.

Mammalian Toxicity Endpoints, including Acute Toxicity, Repeated Dose Toxicity, Ames Mutagenicity, Chromosomal Aberration Testing and Reproductive Toxicity for both PNCB and ONCB have been fulfilled by way of tests that either conformed directly to OECD test guidance or followed test designs similar to OECD guidance. Thus, they have been designated either “1-Reliable without restriction” or “2-Reliable with restrictions”.

An Acute Toxicity study, an Ames test and a Cytogenetics study have been conducted with MNCB and fulfill these Endpoint requirements for this isomer; each of these studies has been designated as either “1-Reliable without restriction” or “2-Reliable with restrictions”. No Repeated Dose Toxicity (of sufficient reliability) or

Reproductive Toxicity studies have been identified for MNCB. Thus, these Endpoints have been filled using the “Read Across” technique for data assessment, since both the ortho and para isomers have been extensively evaluated for these Endpoints.

Based on the conclusions as outlined above on HPV Endpoint assessment, following is a tabular depiction of data availability and testing recommendations for ortho-Chloronitrobenzene (ONCB) (Table 1), meta-Chloronitrobenzene (MNCB) (Table 2) and para-Chloronitrobenzene (PNCB) (Table 3).

Table 1. Test Plan Matrix for ortho-Chloronitrobenzene (ONCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	N	N	R	N	Y	N
Water Solubility	Y	N	N	R	N	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N/Y	Y	N
Stability in Water	Y	N	N	N	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	N	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	N	Y	N
Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	N	Y	N	N	Y	N
Reproductive Toxicity	Y	N	Y	N	N	Y	N
Developmental Toxicity	Y	Y	Y	Y	N	Y	N

Y = Yes; N = No; R = Reputable Reference; - = Not applicable

Table 2. Test Plan Matrix for meta-Chloronitrobenzene (MNCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept-Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	Y	Y	R	N	Y	N
Water Solubility	Y	N	N	R	N	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N/Y	Y	N
Stability in Water	Y	N	N	N	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	N	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	Y	C	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	Y	C	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	Y	Y	Y	N	Y	N
Repeated Dose Toxicity	Y	N	N	Y	N	C	N
Genetic Toxicity – Mutation (Ames)	Y	N	N	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	N	Y	N	N	Y	N
Reproductive Toxicity	N	-	-	-	-	C	N
Developmental Toxicity	N	-	-	-	-	C	N

Y = Yes; N = No; R = Reputable Reference; ; - = Not applicable

C = Read-across from available data on ONCB & PNCB

Table 3. Test Plan Matrix for para-Chloronitrobenzene (PNCB)

	Info. Avail.	OECD	GLP	Other Study	Estimat. Method	Accept- Able ?	Testing Recomm.
PHYSICAL CHEMICAL							
Melting Point	Y	N	N	R	N	Y	N
Boiling Point	Y	N	N	R	N	Y	N
Vapor Pressure	Y	N	N	R	N	Y	N
Partition Coefficient	Y	N	N	R	N	Y	N
Water Solubility	Y	N	N	R	N	Y	N
ENVIRONMENTAL FATE ENDPOINTS							
Photodegradation	Y	N	N	Y	N/Y	Y	N
Stability in Water	Y	Y	Y	-	Y	Y	N
Biodegradation	Y	N	N	Y	N	Y	N
Transport between Environmental Compartments (Fugacity)	Y	N	N	N	Y	Y	N
ECOTOXICITY							
Acute Toxicity to Fish	Y	N	Y	Y	N	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	Y	Y	N	Y	N
Acute Toxicity to Aquatic Plants	Y	N	N	Y	N	Y	N
MAMMALIAN TOXICITY							
Acute Toxicity	Y	N	N	Y	N	Y	N
Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Mutation (Ames)	Y	Y	Y	Y	N	Y	N
Genetic Toxicity – Chromosomal Aberrations	Y	Y	Y	Y	N	Y	N
Reproductive Toxicity	Y	Y	Y	Y	N	Y	N
Developmental Toxicity	Y	Y	Y	Y	N	Y	N

Y = Yes; N = No; R = Reputable Reference; - = Not applicable

V. Data Set Summaries and Evaluations

The key studies used in this assessment to fulfill the HPV requirements for ONCB, MNCB and PNCB have been placed in an Endpoint-specific matrix, and further discussed below. As a number of studies supporting many of these Endpoints exist for each Chloronitrobenzene, key studies were selected based on their representative presentation of data characterization as well as their reliability. Robust Summaries for each study referenced can be found in Section VII of this dossier.

A. Chemical/Physical Properties

A large number of studies are available summarizing the **Physical-Chemical** properties associated with these Chloronitrobenzenes. They can be found in ECB IUCLID Dossiers for o-Chloronitrobenzene (2000), m-Chloronitrobenzene (2000) and p-Chloronitrobenzene (2000). Table 4 contains those values that are considered to best depict the consensus of results found in most key sources used to define the characteristics of each of these Chloronitrobenzenes. They have been obtained from reputable reference books or measured values and cited in peer-reviewed data sources; thus, they are considered “2-Reliable with restrictions”. A Robust Summary has been prepared for each of the references included in Table 4.

In summary, ONCB, MNCB, and PNCB are solid entities at room temperature and possess low vapor pressures. They have a moderate partition coefficient and are moderately soluble in water.

Conclusion: Sufficient data exists to fully characterize the Physicochemical properties of each of these Chloronitrobenzenes. All HPV data requirements for this Endpoint have been met and no further data collection is planned.

Table 4. Selected Physical Properties of Chloronitrobenzenes

Chemical	Boiling Pt. (°C.)	Melting Pt. (° C.)	Vapor Pressure (hPa @ 25 °C)	Water Solubility (mg/L)	Partition Coefficient (Log Kow)
o-Chloronitrobenzene CAS No. 88-73-3	245.7	32.5	0.0575 @ 20°C	198 @ 25°C	2.24
m-Chloronitrobenzene CAS No. 121-73-3	236	46	0.129	273 @ 20°C	2.49
p-Chloronitrobenzene CAS No. 100-00-5	242	83.4	0.1253	189.4 @ 25°C	2.39

B. Environmental Fate and Biodegradation

Semi-Continuous Activated Sludge (SCAS) Biodegradability studies have been conducted to assess the biodegradation potential of ONCB and PNCB; they have been summarized in the Robust Summary section of this Dossier and cited in Table 5 below. While each study was conducted prior to inception of standardized international guidelines for **Biodegradability** testing and GLPs, they followed similar standards for conduct subsequently codified into OECD guideline 302 (Inherent Biodegradation) and GLP documentation. Thus, they are each considered “2-Reliable with restrictions”. An anaerobic bacterial assay with MNCB was selected to fulfill this HPV data requirement as it was well documented and thus also considered “2-Reliable with restrictions”. Supplemental studies summarized in Section VII for each compound confirm the conclusion that Chloronitrobenzenes undergo slow biodegradation in non-adapted soil.

Literature data for ONCB are somewhat conflicting. A report by Zoeteman (1980) indicated that ONCB has an estimated river water half-life in of 3.2 days based on monitoring data from the Rhine River. Another river study, however, indicates that this compound can travel long distances in surface waters (900 miles in the Mississippi River) at concentrations that are explained by simple dilution (Howard, et al. 1976). The SCAS test result of 11 to 48% removal in 24 hours is more consistent with the Zoeteman result. ONCB does not appear to be readily biodegradable but is probably degradable in the environment with time.

The literature indicates that MNCB is resistant to aerobic biodegradation. In aerobic tests using both adapted and unadapted bacteria, Canton et al (1985) found that the half-life was much greater than 4-weeks using either inoculum. The anaerobic test presented in the robust summary shows only that primary degradation takes place under anaerobic conditions. This is not surprising, as the nitro group is expected to undergo facile reduction. The other tests presented in the Robust Summaries are supportive of poor biodegradation. Overall, it can be concluded that MNCB is not readily biodegradable.

PNCB half-life in the Rhine River was also investigated by Zoeteman (1980) who reported a half-life between 0.3 and 3.0 days. The SCAS test result of 34 to 66% removal in 24 hours is consistent with the Zoeteman result. The supplemental studies are also supportive of PNCB being somewhat more easily biodegraded than the ortho isomer. PNCB does not appear to be readily biodegradable but is likely degradable in the environment with time.

A single, comparative study of the photochemical reactions associated with each of the three Chloronitrobenzenes has been summarized in the Robust Summary section of this dossier. It has been classified as “2-Reliable with restrictions”, as it provides useful information, appears well conducted, but did not conform to codified OECD guidelines. Comparative values have been included in Table 5. AOPWIN modeling for this **Photodegradation** Endpoint has also been included to estimate atmospheric indirect

photodegradation. We have incorporated the use of an estimation model (EPIWIN, 2002) for determination of Transport Between Environmental Compartments (**Fugacity**), for all three Chloronitrobenzenes. A Fugacity Level III model was used in each case, and employed measured values, where possible, as recommended by the US EPA. Thus, the estimations derived from each of these models have been classified as “2-Reliable with restrictions”. These estimates have also been included in Table 5 and are cited in the Robust Summary section of this Dossier; data entries used in the Level III fugacity model have been included in the Robust Summaries for validation of output.

No values have been identified to define the **Stability in Water** (hydrolysis) of any of these Chloronitrobenzenes. Further no such values could be calculated using EPIWIN (2002) as each chemical has only aromatic nitro and aromatic chloro functional groups, both of which are listed in Lyman et al. (1990) as Generally Resistant to Hydrolysis. Thermodynamic calculations, however, have been conducted and included in robust summary form to support the hydrolytic stability of these materials in water. Thus, “[t]esting for Stability in Water is not needed for substances generally recognized to have molecular structures or possess only functional groups that are generally known to be resistant to hydrolysis” (OECD, 2002).

Conclusion: Sufficient information exists to characterize the Environmental Fate and Biodegradation of each of these Chloronitrobenzenes. Where experimental data do not exist, use of an estimation model (EPIWIN) recommended by EPA provided necessary information or the rationale lack of need for testing has already been recognized or calculated based on thermodynamics. Thus, all HPV data requirements for this Endpoint are met and no further data collection is planned.

Table 5. Comparison of Environmental Fate Endpoints for Category Members

Chemical	Biodegradation Rate	Stability in Water	Direct Photodegradation (% Disappeared-5 Hr Irradiation)	Fugacity (%)
o-Chloronitrobenzene CAS No. 88-73-3	11-48 % Primary Degrad.(SCAS)	n.d.	66	Air- 6.5 Water- 33.5 Soil- 59.8 Sediment-0.16
m-Chloronitrobenzene CAS No. 121-73-3	50% (anaerobic sediment)	n.d.	89	Air- 8.0 Water- 28.8 Soil- 63.0 Sediment-0.19
p-Chloronitrobenzene CAS No. 100-00-5	31-66% Primary Degrad. (SCAS)	n.d.	96	Air- 9.5 Water- 28.5 Soil- 61.8 Sediment- 0.17

nd. = no data available

To summarize the Environmental fate of these Chloronitrobenzenes, based on Fugacity modeling the members of this Category are expected to be found primarily in the soil and water as main environmental target compartments. None of these chemicals is readily hydrolysable in the environment. They can be abiotically reduced in the presence of natural electron transport mediators and under reducing conditions, but are not Readily Biodegradable. Under conditions of domestic waste treatment, considerable biodegradation is apparent. Estimated Koc values suggest the Chloronitrobenzenes possess moderate mobility in soils (EPIWIN, 2002); slow volatilization is expected to occur, based on their vapor pressures. These chemicals are expected to exist primarily in the vapor phase in the atmosphere where they will degrade slowly by reaction with photochemically producing hydroxyl radicals.

C. Aquatic Toxicity

Several references to acute fish, invertebrate and algal toxicity can be found in the ECB IUCLID documents for ONCB (2000), MNCB (2000) and PNCB (2000). Data presented in Table 6, and summarized in the Robust Summary section VII, depict the level of toxicity generally observed for these Endpoints within the overall dataset. All of the studies selected to fulfill the Acute Fish, Acute Invertebrate and Acute Plant Toxicity Endpoints for ONCB and PNCB were either conducted according to US EPA test guidance (ASTM/EPA) consistent with international guidance or published in a peer-reviewed journal possessing sufficient documentation. Thus, they are considered “2-Reliable with restrictions”. Similarly, a well-documented Acute Fish toxicity study with MNCB, which followed US EPA/ASTM guidance, is also considered “2-Reliable with restrictions”. Two literature articles were found summarizing acute toxicity effects of MNCB in Daphnia and algae. Both purportedly were conducted following OECD or Dutch National testing guidance. Additionally, both articles provided a comparative assessment of all three Chloronitrobenzene isomers considered in this Category. However, neither article provides sufficient detail nor individual data documentation to be assigned a reliability code other than “4- Not assignable” for HPV purposes. A Robust Summary has been completed for each study and included in the Robust Summary Section of all three isomers in Section VII of this Dossier.

Additionally, we have conducted estimation modeling for aquatic toxicity endpoints on all three isomers. Where acceptable measured data were available, these data were used as the critical study, with ECOSAR estimates as supporting information. Where information on measured data were not sufficient (MNCB 48hr. Daphnid EC50 and 96hr. Algal EC50), the ECOSAR results were considered the critical study.

In summary, the empirical data derived from testing and the estimations derived from modeling, support a similar degree of comparative acute aquatic toxicity of all three Chloronitrobenzene isomers to these three aquatic species. Thus, it is reasonable and justifiable to use the “Read Across” technique for fulfilling both the Acute Invertebrate

and Acute Aquatic Plant Toxicity Endpoints for MNCB from empirically derived data available for both ONCB and PNCB.

Conclusion: Sufficient data exists to fully characterize the Acute Aquatic Toxicity properties of each of these Chloronitrobenzenes. All HPV data requirements for this Endpoint have been met with empirical data or through limited and scientifically justified “Read Across” methods such that no further data collection is required for these materials.

Table 6. Comparison of Aquatic toxicity parameters for category members

Chemical	Fish LC 50 (mg/L) (96-hr)	Invertebrate (Daphnia) LC50 (mg/L) (48-hr)	Algae EC50 (mg/L) (48-hr)
o-Chloronitrobenzene CAS No. 88-73-3	30.03 (14-day) (Guppy)	41.0	34.0 (biomass)
m-Chloronitrobenzene CAS No. 121-73-3	18.8 (F. minnow)	44.8 (estim.)	28.8 (96 hour estim.)
p-Chloronitrobenzene CAS No. 100-00-5	6.0 (R. trout)	10.0	8.0 (biomass)

D. Mammalian Toxicity

1.0 Acute Toxicity

Key acute toxicity studies by the oral exposure route were chosen from a number of other acute reports; these results represent acute toxicity values identified from reliable sources. It should be noted that acute toxicity studies with most laboratory animals are not considered sufficiently predictive of the acute hazards of these nitroanilines to humans, due to the resistance observed in lab animals to development of methemoglobinemia. All studies included in Table 7 were conducted specifically or in general agreement with OECD acute toxicity testing guidance and are considered either “1-Reliable without restriction” or “2-Reliable with restrictions”. Other acute toxicity study results are cited in the ECB IUCLID dossiers for ONCB (2000), MNCB (2000) and PNCB (2000).

Table 7. Acute Mammalian Toxicity for Category members

Chemical	Rat Oral LD50 (mg/kg)
o-Chloronitrobenzene CAS NO. 88-73-3	560
m-Chloronitrobenzene CAS No. 121-73-3	400
p-Chloronitrobenzene CAS No. 100-00-5	530

Conclusion: Sufficient data from well-documented studies (Acute Oral Toxicity) exist to meet the Acute Toxicity data set requirements for all members of this Category. Hence, no further acute toxicity testing is planned.

2.0 Repeated Dose Toxicity

PNCB and ONCB have been extensively evaluated in Repeated Dosing studies of various durations and by different exposure routes (ECB IUCLID - PNCB, 2000; ECB IUCLID – ONCB, 2000). Studies conducted in rats for 13 weeks by the inhalation exposure route with ONCB and PNCB, each consistent with OECD Test Guideline 413, have been selected to fulfill the requirements for this HPV Endpoint. Each of those studies is summarized in Table 8, is considered “1-Reliable without restriction” and has been included in the Robust Summary section of this dossier. Additional Repeated Dose rat inhalation studies of a shorter duration (4-weeks), have been included as Supplemental information in Table 8 and summarized in the Robust Summary section of this dossier, as they are useful for comparative purposes. Additionally, it should be noted that other Repeated Oral Dose studies with PNCB are available and have previously been submitted to EPA and are cited in the ECB IUCLID – PNCB (2000). These studies include: a chronic/carcinogenic oral rat study (Nair et al, 1989), a 13-week oral toxicity study in rats (Solutia, 1979).

No adequately reported Repeated Dose studies were found for MNCB after an extensive literature search as well as review of its ECB IUCLID (2000) document. However, the summary of a series of studies comparing MNCB repeated dose toxicity with that of PNCB and ONCB was found (Davydova, 1967). It has been included in this discussion as it provides some useful Supplemental information. Due to its inclusion as only summary data, it has been assigned a Reliability classification of “4-Not Assignable”. While included in the Robust Summary section of this dossier, it has not been included in Table 8.

Conclusion: The Repeated Dose HPV Endpoint for both PNCB and ONCB are complete with selection of a 13-week inhalation study in rats for each chemical, as each meets OECD Test Guideline 413; thus, no further testing is needed.

It is scientifically justifiable to consider completion of the Repeated Dose HPV Endpoint for MNCB through use of the “Read Across” technique for data assessment, based on 1) similarity of structure, i.e. it is one of three nitrobenzene isomers considered in this dossier, 2) substantive and fully adequate testing for this Endpoint already exists for the other two isomeric forms, PNCB and ONCB, 3) there is a known, identical mode of action associated with all three isomers (methemoglobinemia) and 4) a consistent pattern of repeated dose toxicity has been established among the three isomers. Clinical observations, serum chemistry changes, organ weight differences and histopathological findings associated with PNCB and ONCB were related to methemoglobin formation and

compensatory processes that occurred as a result. The single Supplemental study found in the literature with MNCB characterized its repeated dose toxicity as fully comparable with that seen with PNCB and ONCB. However, the degree of potency of MNCB was characterized as closer to the more toxic isomer, PNCB, rather than ONCB, the lesser toxic isomer.

Conclusion: “Read Across” methodology, based on the use of reliable data from PNCB and ONCB, is scientifically justified to adequately characterize the Repeated Dose hazards associated with MNCB. Thus, the requirements for the Repeated Dose HPV Endpoint for MNCB are complete and no further, unnecessary animal testing is warranted.

Table 8. Repeated Dose Toxicity Studies with Category Members

Chemical	Study Type	Dosages	Histopathology	Hematology/Clinical Findings
o-Chloronitrobenzene CAS NO. 88-73-3	13-Week Rat Inhalation 10M/10F/group F344 rats	18 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Spleen-congestion Kidney-hemosiderosis Kidney, Liver,Spleen Wt	MET, RETIC, SDH, LB,ALT, AP, B acids HCT, HGB, RBC, PLAT
		9 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Kidney-hemosiderosis Kidney, Liver Wt	MET, RETIC, SDH, LB,ALT, AP, B acids HCT, HGB, RBC, PLAT, MCHC/MCH(F)
		4.5 ppm	Respir. Epithel.-hyperplasia Liver-basophilia Kidney-hemosiderosis Spleen Wt	MET, SDH, ALB,ALT, B acids HCT, HGB, RBC
		2.3 ppm	Respir. Epithel.-hyperplasia Liver Wt	MET, SDH, ALB,ALT, B acids; HCT
		1.1 ppm	Respir. Epithel.-hyperplasia	MET
o-Chloronitrobenzene CAS NO. 88-73-3	4-Week Rat Inhalation 15M/15F/group S-D Rats	60 mg/m ³ (~9.3 ppm)	Spleen-Extramed. Hematopoiesis & hemosiderosis Liver, Kidney, & Spleen Wt	MET, RET HCT, HGB, RBC
		30	Spleen-Extramed.	MET

		mg/m ³ (~4.6 ppm)	Hematopoiesis & hemosiderosis Liver, Kidney, & Spleen Wt	HCT (F), HGB (F), RBC (F)
		10 mg/m ³ (~1.5 ppm)	Liver Wt (M)	-
m-Chloronitro- benzene CAS No.121-73-3		No Data		
p-Chloronitro- benzene (PNCB) CAS No.100-00-5	13-Week Rat Inhalation 10M/10F/group F344 rats	24 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis- hyperplasia Testes - atrophy Liver, Spleen, Heart, Thymus, Testes weights	MET, RET, MCH, n-RBC, SDH, B acids HCT, RBC, HGB, AP, GLOB, ALT, TPROT
		12 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis- hyperplasia Liver, Spleen, Heart weights	MET, RET, n- RBC, SDH, B acids HCT, RBC, HGB, AP, GLOB, ALT, TPROT
		6 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis/fibrosis- hyperplasia Liver, Spleen weights	MET, RET, n- RBC, SDH (F), B acids (M) HCT, RBC, AP, GLOB, ALT, TPROT
		3 ppm	Renal-hyaline droplets (M only) Spleen & B. Marrow-Hematopoietic cell prolif. Hardarian gland-proliferation Spleen & Liver-hemosiderosis Liver, Spleen weights	MET, RET, n- RBC, B acids (M) HCT, HGB, RBC, ALT (M)
		1.5 ppm	Renal-hyaline droplets (M only) Spleen -hemosiderosis	MET, RET, n- RBC HCT, HGB, RBC,

				ALT (M)
<p>p-Chloronitrobenzene (PNCB) CAS No.100-00-5</p>	<p>4-Week Rat Inhalation 15M & 15F/group S-D rats</p>	<p>45 mg/m³ (~ 7 ppm)</p>	<p>Spleen-congestion & hemosiderosis & Extramedullary hematopoiesis Liver & Spleen weight</p>	<p>MET HCT, HGB, RBC</p>
		<p>15 mg/m³ (~ 2.3 ppm)</p>	<p>Spleen – hemosiderosis Liver weight (F)</p>	<p>MET HCT, HGB, RBC</p>
		<p>5 mg/m³ (~ 0.8 ppm)</p>	<p>Spleen - hemosiderosis</p>	<p>HCT, HGB, RBC</p>

3.0 Mutagenicity and Chromosomal Aberrations

Ames Test

For each of the three Chloronitrobenzene isomers, a key point mutation study has been selected to fulfill this HPV Endpoint. Both the ONCB and PNCB studies were conducted according to GLPs and conformed to OECD Test Guideline 471 and thus are considered “1-Reliable without restriction”. The study with MNCB was well documented but conducted prior to OECD Test Guideline codification and thus is considered “2-Reliable with restrictions”. Each study has been cited in Table 9 and summarized in the Robust Study section of this Dossier. Additional Ames test assays are reported in the ECB IUCLID for ONCB (2000), MNCB (2000), and PNCB (2000).

Weak positive responses were seen in Salmonella with ONCB and PNCB but not MNCB. Both ONCB and PNCB have been consistently inactive (negative) in *in vitro* assays using mammalian cell lines, including the CHO/HGPRT assay (Solutia 1982a, 1983a), the UDS Rat Hepatocyte Culture assay (Solutia 1983b, 1984) and a rat hepatocyte DNA repair assay with PNCB (Solutia, 1982b). PNCB was positive only with metabolic activation in

the Mouse Lymphoma assay (Solutia, 1981). Neither PNCB nor ONCB induced sex-linked recessive lethal germ cell mutations in an *in vivo*, secondary tier mutation assay (NTP, 1993).

Conclusion: The Ames Test Category Endpoint for each of the Chloronitrobenzenes has been met and no further testing should be considered for the gene point mutation endpoint for this chemical.

Table 9. Genetic Toxicity of Category Members

Chemical	Ames Test- TA98, 100, 1535, 1537 +/- activation	Cytogenetics In Vitro (CHO Cells)	Cytogenetics In Vivo
o-Chloronitrobenzene CAS NO. 88-73-3	Positive – TA100 w S-9 Negative – TA100 w/o S-9 Negative -. w & w/o S-9. TA98, TA1535, TA1537	Weak Positive- w S-9 Negative – w/o S-9	n.d.
m-Chloronitrobenzene CAS No. 121-73-3	Negative – TA100, TA98, TA1535, TA1537, TA1538 w and w/o S-9	Negative - w & w/o S-9	n.d.
p-Chloronitrobenzene CAS No, 100-00-5	Positive – TA1535 w/o S-9 Ambiguous- TA1535 w S-9 Negative – TA98, TA1537, TA100 w and w/o S-9	Weak Positive – w & w/o S-9	Negative

n.d. = no data

Chromosomal Aberrations -

Three *in vitro* CHO cell chromosomal aberration studies sponsored by the US NTP program, each with a different Chloronitrobenzene isomer, have been conducted following a study design similar to, but not identical with, OECD Test guideline 473. Each study was well documented and followed GLPs and thus is considered to be “2-Reliable with restrictions”. These studies have been used to fulfill this HPV Endpoint for ONCB and MNCB. However, while the CHO cell study could be used to support this Endpoint for PNCB, a secondary tier, *in vivo* Chromosomal Aberration Test (classified “2-Reliable with restrictions”) has been chosen as the key HPV study for this chemical.

Conclusion: On the basis of reliable in vitro (ONCB and MNCB) and in vivo (PNCB) Chromosomal Aberration Assays available for each of these Chloronitrobenzenes, no additional testing is needed to fulfill this HPV Endpoint.

4.0 Reproductive and Developmental Toxicity

PNCB, the most toxic chemical in this Chloronitrobenzene group, has undergone testing for developmental toxicity in two species (rat and rabbit) and has been evaluated both in a rat Two-Generation Reproduction study and a mouse Continuous Breeding study. Each of these studies have been assessed as “1-Valid without restriction” as they fully met OECD testing guidelines (or standardized methodology as in the case of the Continuous Breeding study) and GLP guidance. The Two Generation Rat Reproduction study has been selected as the key study to fulfill the reproductive toxicity endpoint for PNCB as its design is considered more conventional than the Continuous Breeding study. The rat developmental toxicity study has been marked as key for the developmental toxicity endpoint and the rabbit study is included as Supplemental information. Each of these adequately conducted studies has been summarized in Table 10 and Robust Summaries have been developed.

ONCB has been evaluated in a comparative (to PNCB) rat developmental toxicity study filling this HPV endpoint. This study has also been evaluated as being “1-Valid without restriction” and has been summarized in Table 10. Additionally, relative to the reproductive toxicity endpoint, it has been tested in a mouse Continuous Breeding study, as has PNCB. As the Continuous Breeding study was conducted in accord with standardized testing methodology for this reproduction study and under GLPs, it has been classified as “1-Reliable without restriction” and fulfills the Reproductive Toxicity HPV Endpoint for ONCB. Robust Summaries for each study can be found in Section VII of this Dossier.

To summarize the available information on these two Chloronitrobenzene isomers, ONCB was judged “not to be a reproductive toxicant, even in the presence of systemic toxicity in Swiss CD-1 mice” (NTP, 1993). PNCB produced no effects on reproductive toxicity parameters through 2 generations in rats up to a level (5 mg/kg/d) known to produce significant systemic toxicity (Nair et al, 1989). Significant and progressive deficits in infertility in the FO generation and reduced weight gains in F1 and F2 pups were seen in mice during the Continuous Breeding study and may have been related to methemoglobin-related hypoxia associated with cyanosis observed at PNCB test levels. Developmental toxicity was seen only at the highest dose tested in rats with PNCB, and thus was judged to not to have a primary effect on fetal development. ONCB produced no developmental toxicity when evaluated in rats even at maternally toxic levels.

No Reproductive Toxicity or Developmental Toxicity studies have been identified with MNCB. However, we believe sufficient data exists in this Category to obviate the need for further evaluation of MNCB, based on the similarity of mammalian toxicity of this group of Chloronitrobenzene isomers and through use of the

corresponding reproductive toxicity data available on both PNCB and ONCB. A “Read Across” approach, using the PNCB and ONCB reproductive studies in rats and mice, has been used to fulfill the Reproductive Toxicity HPV Endpoint for MNCB. As there are differences noted in potency and effects seen between PNCB (greater toxicity) and ONCB (lesser toxicity)(see below), we believe it appropriate to associate similarity of effects projected with MNCB with those of PNCB. This provides both a more conservative approach to assignment of effects as well as the most scientifically justifiable, as human experience and repeated dose testing in animals support closer analogy of response between MNCB and PNCB than between MNCB and ONCB.

Thus, we conclude that use of all available data in the Category approach, along with key studies with ONCB and PNCB, allows this HPV Endpoint to be completed without further unnecessary testing of MNCB.

Table 10. Summary of Developmental Toxicity and Reproduction Studies with Category Members

Chemical	Study Type/Species	Dosage	Observations	Conclusion
o-Chloronitrobenzene (ONCB) CAS NO. 88-73-3	Rat Teratology – Gavage 25 /group	150 mg/kg	Maternal Toxicity: 6/25 early deaths	no further investigation
		100 mg/kg	Maternal Toxicity: Body wt gain Food consump. 1 death; No terata, embryotox or fetotox	NOEL for Embryotoxicity, Fetotoxicity, Teratogenicity
		75 mg/kg	Maternal tox; Food consump. 1 death	
		25 mg/kg	No findings	NOEL for Maternal toxicity
o-Chloronitrobenzene (ONCB) CAS NO. 88-73-3	Mouse Continuous Breeding	160 mg/kg	Methem in FO & F1 FO (M/F) spleen wts F1(m) spleen and liver wts ; sem. Vesic.Wt F1 (Final litter) M/F pup wt.	NOEL – fertility Indices. Reproductive NOAEL was 320 mg/kg
		80 mg/kg	FO (M/F) spleen wts F1 (Final litter) M/F pup wt.	

		40 mg/kg	F1 (Final litter) female pup wt.	
m-Chloronitrobenzene (MNCB) CAS NO. 121-73-3	No studies found			
p-Chloronitrobenzene (PNCB) CAS No. 100-00-5	Rat Teratology – Gavage 25/group	45 mg/kg 15 mg/kg 5 mg/kg	Maternal toxicity: Body wt. Gain Spleen wt. Embryotoxicity: Resorptions Fetotoxicity: Fetal wts. Terata: skeletal Maternal toxicity: Body wt. Gain Spleen wt. No terata, embryo- or fetotoxicity No findings	NOEL for teratogenicity, fetotoxicity and embryotoxicity Maternal toxicity NOEL
p-Chloronitrobenzene (PNCB) CAS No. 100-00-5	Rabbit Teratology - Gavage 18/group	125 mg/kg 75 mg/kg 25 mg/kg	Maternal Toxicity: Deaths (7/18) Physical changes Maternal toxicity: Physical changes No findings	NOEL for Terata, fetotoxicity, and embryotoxicity NOAEL for Maternal Toxicity Unequivocal NOEL for Maternal Toxicity
p-Chloronitrobenzene (PNCB) CAS No. 100-00-5	Two-generation Rat Gavage Reproduction Study 15 males/30 females per group in F0 and F1 generations	5 mg/kg 0.7 mg/kg	Parental toxicity: Histopathology consistent with methemoglobinemia F0/F1: all mating indices judged normal No findings	NOEL for all reproductive endpoints NOEL: Maternal & paternal toxicity

		0.1 mg/kg	No findings	
p-Chloronitro- benzene (PNCB) CAS No. 100-00-5	Mouse	250 mg/kg	Most animals visibly cyanotic FO-Fertility (after 1 st litter) F1-spleen and liver wt ; estrus cycle F1 & F2 pup wt F2 pup survival and wts	
	Continuous Breeding			
		125 mg/kg	FO-Fertility (after 1 st litter) F1 & F2 pup wt	
		62.5 mg/kg	FO-Fertility (after 1 st litter) F1 male pup wt	

In summary, as seen previously in sections dealing with acute and repeated dose testing for mammalian toxicity endpoints, PNCB has proven to produce the more significant comparative toxicity, hence the lower dosages used in the developmental toxicity studies listed. Albeit tested at lower dosages, only PNCB exhibited significant developmental toxicity in the comparative rat studies. Severe maternal toxicity, along with embryotoxicity, fetotoxicity and frank malformations were observed at the highest dosage tested. Only maternal toxicity and no embryotoxicity or fetotoxicity was observed at the mid dosage employed while the low dose selected was without treatment-related effect. As developmental effects were noted only at a dosage that produced significant maternal toxicity, PNCB is not considered to cause a primary effect on fetal development.

PNCB was toxic to rabbits in a developmental toxicity study (Nair et al, 1985). Frank maternal toxicity, including deaths, was observed at the highest dose tested, thus rendering determination of developmental toxicity impractical at this dosage level. There was no evidence of developmental toxicity observed at either of the two lower test levels used in this study.

ONCB, on the other hand, produced substantive maternal toxicity in rats at 100 mg/kg, but produced no evidence of embryotoxicity, fetotoxicity or teratogenicity even at this level.

PNCB produced no evidence of adverse reproductive performance, including mating, fertility and pregnancy, littering or pup survival and development, in a Two-Generation rat Reproduction study using a top dosage which produced significant maternal toxicity (increased spleen weight, anemia, elevated blood methemoglobin

levels) related to methemoglobinemia following chronic dosing (Nair et al, 1989). PNCB, but not ONCB, affected reproductive outcomes in mice exposed during a series of continuous breeding cycles.

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VII. ROBUST STUDY SUMMARIES

Appended