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Assessment of the genotoxic and carcinogenic risks of *p*-nitrophenol when it is present as an impurity in a drug product

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ABSTRACT

According to the 2008 US FDA (draft) and 2006 EMEA guidance documents for genotoxic impurities, an impurity that is positive in an in vitro genotoxicity study, in the absence of in vivo genotoxicity or carcinogenicity data, should be treated as genotoxic and typically controlled to 1.5 μg/day for chronic use. For *p*-nitrophenol (PNP), existing study results (i.e., positive in vitro clastogenicity in mammalian cells, no information on its in vivo genotoxicity, and negative with respect to carcinogenicity in a dermal mouse study with no confirmation of systemic exposure) indicated that it should be considered genotoxic and exposure as a drug impurity limited. Therefore, to more completely characterize the genotoxic potential of PNP (consistent with the guidance documents), in vivo mouse micronucleus and dermal pharmacokinetic bridging studies were conducted. In the micronucleus study, PNP was negative, demonstrating that the reported in vitro clastogenicity is not present in vivo. In the pharmacokinetic study, PNP was well absorbed dermally, validating the negative dermal carcinogenicity assessment. These results indicate that PNP should be considered a non-genotoxic impurity and, as a drug impurity, a threshold limit of ⩽4 mg/day would be set (per ICH Q3C). This threshold limit is higher than the EPA reference dose (listed in the 2006 Edition of the Drinking Water Standards and Health Advisories), so if present at such levels, the specification limits for PNP should be determined on a case-by-case basis, based on risk-benefit.

1. Introduction

p-Nitrophenol (PNP) is a synthetic chemical that is used as a starting material for pharmaceutical products and dyes and as a fungicide. As such, the toxicity and safety profile of PNP has been reviewed by several health agencies including the Agency for Toxic Substances and Disease Registry—US Public Health Service in 1992[1], the National Technology Program in 1993[2] and the EPA in 1998[3]. The EPA granted registration for PNP in 1980 as a fungicide for incorporation into leather for military use, at a concentration not to exceed 0.7% on a basis of dry finished leather weight. In 1983 this registration was amended to add the use of the product for incorporation into cork insulation for military use. The safety reviews by the health authorities are based on data from general, reproductive and in vitro genetic toxicology studies as well as a mouse dermal carcinogenicity study.

Although a significant amount of information is available for PNP, gaps in the studies conducted preclude a definitive assessment of genotoxic potential, which is critical to meet stringent regulatory health authority requirements for impurities in drug product (EMEA, 2006; FDA, 2008). In recent years, there has been increased regulatory focus on impurities in drug products, requiring identification and control of genotoxic impurities in drug products to very low levels, which has added a significant burden of proof to clearly define genotoxic potential of impurities that are part of the manufacturing process. The results from previous studies and reviews of PNP toxicity, as well as additional studies to better define PNP risk, are summarized in the sections that follow.

1.1. Alerting structural features

Due to its nitro aromatic structure, PNP warrants scrutiny for genotoxicity, since several nitro aromatics have been shown to be genotoxic (Huang et al., 1996; Klopman and Rosenkranz, 1984; Rosenkranz and Mermelstein, 1983). Substituted nitrobenzenes can undergo reduction to arylhydroxylamines or hydroxamic esters, which contain electrophilic nitrogen atoms that can react with nucleophilic centers in cellular DNA (e.g. the C8 position of guanine residues in DNA) (Huang et al., 1996).

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[1] http://www.atsdr.cdc.gov/toxprofiles/tp50-p.pdf.
[2] http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr417.pdf.
[3] http://www.epa.gov/oppsrrd1/REDs/2465red.pdf.

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Table 1
Mammalian toxicity of PNP (Source: http://www.epa.gov/chemrtk/pubs/summaries/4ntrophn/c14390.pdf).

Acute toxicity		Repeat dose toxicity			Reproductive toxicity
Oral LD50 (rat) 230 mg/kg	Dermal LD50 (rabbit) >5000 mg/kg	Oral 90-day (rat) NOEL 25 mg/kg/day	Inhalation 28-day (rat) NOEL 5 mg/m^3	Dermal chronic (mouse) NOEL (systemic toxicity/ carcinogenicity) 160 mg/kg/ day	Dermal 2-generations (rat) NOEL (maternal-systemic) 250 mg/ kg/day NOEL (reproductive toxicity) 250 mg/kg/day

1.2. In vitro metabolism

In vitro and in vivo non-clinical metabolism studies suggest that glucuronide or sulphate conjugation is a major metabolic route for PNP (Diamond et al., 1982; Machida et al., 1982; Meerman et al., 1987). Other possible routes of metabolism include nitro reduction to form *p*-aminophenol or phenyl oxidation to form *p*-nitrocatechol. For a more detailed review of the in vitro metabolism of PNP, the reader is referred to a 1992 review by the Agency for Toxic Substances and Disease Registry—US Public Health Service (ATDSR, 1992).

1.3. Previous general toxicology studies

The results from previously conducted general toxicology studies are summarized in Table 1. Since the NOELs in the general toxicity studies were greater than or equal to those in the genetic toxicology and carcinogenicity studies (see sections that follow), the exposure margins with respect to the genotoxicity and carcinogenicity studies were considered to be more appropriate for determining acceptable levels of human exposure.

1.4. Previous genetic toxicology studies

A summary of the previous genetic toxicology studies are presented in Table 2 and discussed in more detail in the sections that follow.

1.4.1. Reverse mutation tests in bacterial cells

PNP has been evaluated in several Ames tests. In general, tests with the various strains of *Salmonella typhimurium* in the absence and presence of metabolic activation with rat liver S9 have been negative (Buselmaier et al., 1972; Commoner, 1976; Haworth et al., 1983; McCann et al., 1975; Shimizu and Yano, 1986; Suzuki et al., 1983) and high concentrations (up to 10 mg/plate) have been used. However, in the IUCLID Dataset (2000) one Ames test was reported positive, although no reference or details were given. Some tests have also been conducted in strains of *E. coli*. Again according to the IUCLID Dataset, one test with *E. coli* strain WP2*uvr*A was positive while another test with *E. coli strain* WP2*uvr*A pKM101 was negative. No reference or details were given.

According to the IUCLID Dataset, DNA repair tests have been performed in *B. subtilis* (positive) and *E. coli* (negative), but no details were given and published versions of these tests have not been found, so the reliability of the data cannot be judged. Overall it is considered that PNP is not mutagenic to bacteria. This is consistent with statements from a number of expert bodies:

- In their Technical Report 147, NTP stated that "PNP does not appear to be genotoxic".
- In the US Public Health Service Toxicology Profile for nitrophenols (1992) it is stated that the overall evidence indicates that *p*-nitrophenol is not mutagenic in bacteria.
- In the US EPA Re-registration Eligibility Decision for *p*-nitrophenol (1998) it is stated that "Overall the data presented ... indicate that while the aromatic nitro group on *p*-nitrophenol is a structural alert for DNA reactivity, the test substance is not a mutagen for bacteria".

The lack of mutagenic potential in bacteria is important in the assessment of PNP. Although, of the available in vitro tests, the Ames test is not the most predictive for rodent carcinogenicity (see Kirkland et al., 2005), the reasons for false negative results are well known. Carcinogenic substances which require eukaryotic receptors or processes for their mode of action, and those that cause large DNA deletions (i.e. would delete the target gene and

Table 2
Summary of results from previous genetic toxicology studies on PNP.

Assay	PNP concentration (s)	Result(s)	Conclusion(s)	Source(s)
Ames battery in presence and absence of S9	High (up to 10 mg/plate)	All negative except one Ames test in IUCLID Dataset with no details given	Not mutagenic in bacteria	(Buselmaier et al., 1972; Commoner, 1976; Haworth et al., 1983; McCann et al., 1975; Shimizu and Yano, 1986; Suzuki et al., 1983)
Mouse lymphoma only in presence of S9	High (up to 782 μg/mL)	Equivocal results—toxicity endpoints not reached and mutation frequency comparable to control	Not mutagenic in mouse lymphoma cells	(Amacher and Turner, 1982)
CHO hypoxanthine–guanine phosphoribosyl transferase activity (*hgprt*)	High (up to 800 μg/mL)	All negative; some cytotoxicity with S9	Does not induce *hgprt* mutations in CHO cells	(Oberly et al., 1990)
Chromosome aberration studies in CHO and CHL cells	High (750–2000 μg/mL)	Induced chromosomal aberrations at non-toxic concentrations in the presence of metabolic activation	Causes chromosome aberrations in mammalian cells	MRID 4220901 in the US EPA Re-registration Eligibility Decision for *p*-nitrophenol, 1998 Unpublished Japanese study: study director Noda, performed at Research Institute on Safety for Animal and Biological Sciences)(Noda, xxxx)
Unscheduled DNA synthesis (UDS) in rat hepatocytes	Low to moderate (13.9 μg/mL)	No unscheduled DNA synthesis observed	Negative	(Probst et al., 1981)
DNA damage in V79 cells using the Comet assay	High (1390 μg/mL)	No comets observed	Negative	(Hartmann and Speit, 1997)