Available online at www.sciencedirect.com
ScienceDirect
Mutation Research 608 (2006) 58–71
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

In vitro genotoxicity of *para*-phenylenediamine and its *N*-monoacetyl or *N*,*N*′-diacetyl metabolites

Jean-Luc Garrigue [a,*], Mark Ballantyne [b], Tirukalikundram Kumaravel [b], Mel Lloyd [b], Gerhard J. Nohynek [a], David Kirkland [b], Hervé Toutain [a]

[a] *L'Oréal Research and Development, Worldwide Safety Department, 92665 Asnières-sur-Seine Cedex, France*
[b] *COVANCE Laboratories Ltd., Otley Road, Harrogate, North Yorkshire HG3 1PY, United Kingdom*

Received 26 January 2006; received in revised form 21 April 2006; accepted 4 May 2006
Available online 30 June 2006

Abstract

para-Phenylenediamine (PPD), a widely used ingredient of oxidative hair dyes, is converted by human hepatocytes and in the human epidermis, or after topical application to rats, to its *N*-monoacetylated (MAPPD) and/or *N*,*N*′-diacetylated (DAPPD) derivatives. We investigated *in vitro* genotoxic properties of PPD, MAPPD and DAPPD in the Ames test, the micronucleus test (MNT) in human lymphocytes and the mouse lymphoma assay (*Hprt* locus, PPD only). Given that MAPPD and DAPPD are actual human skin and hepatic metabolites of PPD and represent the substances to which humans are systemically exposed, they were tested in the absence of metabolic activation.

In the Ames test, PPD was slightly mutagenic in *Salmonella typhimurium* strain TA98 in the presence of a rat liver metabolic activation system (S-9), whereas MAPPD and DAPPD were negative in all strains. When tested up to toxic doses, PPD did not induce mutation at the *Hprt* locus of L5178Y mouse lymphoma cells in two independent experiments, either in the absence or presence of S-9, suggesting that PPD is non-mutagenic in mammalian cells. In the *in vitro* micronucleus test, PPD induced micronuclei (MN) in cultured human peripheral blood lymphocytes (HL) in the presence of S-9, when tested following 24-h PHA stimulation. No increases in MN frequency were observed in the absence of S-9, when tested following 24-h PHA stimulation. However, PPD induced MN both in the absence and presence of metabolic activation, when tested following 48-h PHA stimulation. In contrast, MAPPD and DAPPD did not induce MN in HL when tested up to 10 mM concentrations or to their limit of solubility, respectively, after either 24- or 48-h stimulation. In conclusion, the results of the Ames and MN tests confirm that PPD has a slight genotoxic potential *in vitro*, although it was non-mutagenic in mammalian cells. Given that MAPPD and DAPPD were negative in the Ames and the MN tests, these acetylated conversion products are considered to be detoxified metabolites that are biologically less reactive than the parent molecule PPD.

Keywords: *para*-Phenylenediamine; *N*-acetyl-*p*-phenylenediamine; *N*,*N*′-Diacetyl-*p*-phenylenediamine; Ames test; *In vitro* micronucleus test; Mouse lymphoma assay

Abbreviations: PPD, *para*-phenylenediamine (*p*-phenylenediamine, 1,4-benzenediamine); MAPPD, *N*-monoacetyl-*para*-phenylenediamine (*N*-acetyl-*p*-phenylenediamine, 4-aminoacetanilide); DAPPD, *N*,*N*′-diacetyl-*para*-phenylenediamine (*N*,*N*′-*p*-phenylenebisacetamide); NAT1, NAT2, *N*-acetyltransferase-1 or -2; AA, arylamine; MNT, *in vitro* micronucleus test; MN, micronuclei; HL, human lymphocytes; MLA, mouse lymphoma assay; *Hprt*, hypoxanthine-guanine phospho-ribosyl transferase; 2-AF, 2-aminofluorene

* Corresponding author at: L'Oréal R&D, 25–29 quail Aulagnier, 92665 Asnières-sur-Seine, Cedex, France. Tel.: +33 1 47 56 85 26; fax: +33 1 47 56 84 02.
E-mail address: jlgarrigue@rd.loreal.com (J.-L. Garrigue).

1383-5718/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2006.05.001

1. Introduction

Arylamines (AAs) include chemicals that are widely used in the pharmaceutical, chemical, rubber, dye, photographic or cosmetic industries and represent a large chemical family with a wide spectrum of toxicological properties. Although many low-molecular weight AAs are relatively non-toxic and non-carcinogenic [1,2], this chemical class includes known human carcinogens such as benzidine, 4-aminobiphenyl or 2-naphthylamine, which were recognised as early as the 19th century to produce bladder cancer in exposed workers of the dye or textile industries [3]. AAs such as *para*-phenylenediamine (PPD), *para*-toluenediamine (PTD) or *para*-aminophenol (PAP), are important ingredients of oxidative hair dyes (Fig. 1) and thus have considerable potential to produce human skin and, possibly, systemic exposure. Therefore, considerable attention of toxicologists and epidemiologists has focused on potential carcinogenic effects of AA-type hair dyes. Although a single case-control epidemiological investigation suggested an association between use of hair dyes and bladder cancer [4], most other investigations, including large cohort studies [5] or meta-analysis [6], showed no indications for a causal association. Moreover, the weight of evidence of animal studies suggests that PPD or PTD are non-carcinogenic [7,8], which has been further supported by the results of a recent investigation that showed that neither human cytochromes nor human hepatocytes convert PPD to electrophilic and potentially carcinogenic metabolites, whereas they readily convert the carcinogen 2-aminofluorene (2-AF) to a number of hydroxylated metabolites, including the *N*-hydroxylated precarcinogen [9].

Mammalian epidermis has a high *N*-acetyl transferase 1 (NAT1)-mediated metabolic capacity [10–12] and is able to transform certain AAs to *N*-acetylated metabolites [13]. For example, human epidermis as well as

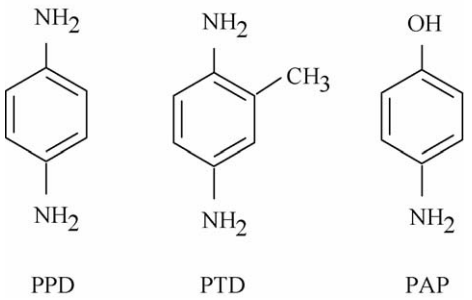

Fig. 1. Chemical structures of important arylamine hair dye ingredients: *para*-phenylenediamine (PPD), *para*-toluenediamine (PTD) and *para*-aminophenol (PAP).

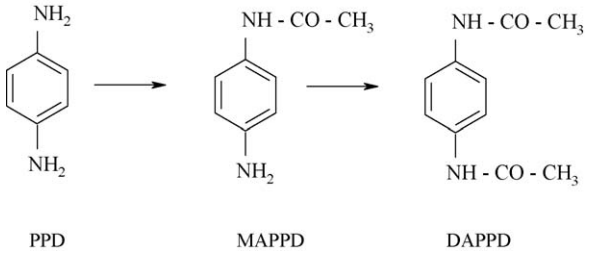

Fig. 2. Biotransformation of *para*-phenylenediamine (PPD) in human epidermis and hepatocytes or after topical administration to rats to *N*-mono- (MAPPD) or *N*,*N*′-di-acetylated (DAPPD) derivatives [9,12–14].

human hepatocytes converted *para*-phenylenediamine (PPD) to its *N*-mono- (MAPPD) and *N*,*N*′-di-acetylated (DAPPD) metabolites [9,14] (Fig. 2). Plasma of rats after a 24-h dermal application of PPD contained only DAPPD [15]. Studies in human volunteers demonstrated that hair dyeing with a [^{14}C]-PPD-containing oxidative hair dye produced low human exposure levels (plasma C_{max} at 0.087 μg/mL [^{14}C]-PPD-equivalents), suggesting a minimal systemic exposure, whereas the urine of exposed subjects principally contained MAPPD and DAPPD [16,17]. A similar metabolic conversion was observed for *para*-aminophenol (PAP), which was metabolised by human epidermis or hepatocytes, or after topical application to pig or rat skin, to its acetylated derivative, i.e. the common drug paracetamol [14,15].

Although other metabolic pathways cannot be excluded with complete certainty, all available data in human hepatocytes, skin or urine, and in rat plasma, showed only the presence of acetylated derivatives of PPD. Given that the available evidence suggests that topical administration of PPD produces systemic exposure of the human organism to small amounts of acetylated metabolites, the question may be raised whether these compounds represent a systemic hazard to human health. Although acetylation of AAs is generally considered to be a detoxification reaction [18], this is not necessarily true for all substances or in all potential target tissues [19,20]. It is therefore of major interest to investigate the potential toxicity of some representative acetylated metabolites. Taking into account that PPD was reported to be positive in the Ames test and in *in vitro* chromosome aberration assays [21], these tests provide ideal tools for comparison of the genotoxic potential of the parent molecule, PPD, with that of its conversion products MAPPD and DAPPD. To this end, we investigated in parallel PPD, MAPPD and DAPPD in the Ames and *in vitro* micronucleus tests. As the acetylated forms of PPD were the only metabolites found in human epidermis and human hepatocytes, and thus represent the actual sub-