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# Dermal penetration and resorption of beta-naphthylamine and *N*-phenyl-beta-naphthylamine from lubricants in an *ex vivo* human skin model

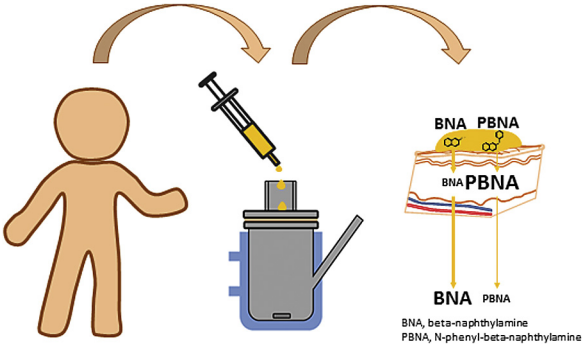
Kathrin Dennerlein [a], Thomas Göen [a], Melanie Zobel [a], Anja M. Boos [b], Hans Drexler [a], Sonja Kilo [a, *]

[a] Institute and Out-Patient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Schillerstrasse 25/29, 91054 Erlangen, Germany
[b] Department of Plastic and Hand Surgery, University Hospital of Erlangen, Friedrich-Alexander University of Erlangen-Nürnberg (FAU), Krankenhausstrasse 12, 91054 Erlangen, Germany

## HIGHLIGHTS

- New insights into dermal uptake of environmental and occupational relevant AA's.
- Workplace exposure scenarios for investigation of dermal resorption of AA's *ex vivo*.
- For the first time intra- and transdermal penetration of PBNA has been shown.
- Characterization of dermal penetration of carcinogenic BNA through human skin.
- Formulation and co-exposure have a relevant impact on dermal uptake of both AA's.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Dermal Penetration of aromatic amines (AA's), often suspected or known to be carcinogenic, can play an important role in the overall human exposure. However, information on penetration of certain AA's is poor and inconsistent. Penetration of the former lubricant additive *N*-phenyl-beta-naphthylamine (PBNA) and its contaminant beta-naphthylamine (BNA) a known carcinogen was investigated and the influence of formulation and co-application characterized.

Percutaneous penetration of BNA and PBNA through freshly excised human skin ($n = 8$; 48 h) was investigated using an *ex vivo* diffusion cell model. Both AA's were applied in a technical-conform lubricant or dissolved in hexane. The amount of BNA and PBNA applied to skin was 0.52 and 259 μg/0.64 $cm^2$. The analytical determination of AA's was performed by GC-MS.

Both, BNA and PBNA penetrated through human skin (38 vs. 5% of applied dose). In contrast to BNA, the percutaneous penetration of PBNA continued beyond the end of exposure. Co-exposure of both AA's increased the intradermal uptake of BNA and PBNA ($p < 0.05$). Exposure in lubricant showed the least overall penetration (2.9 and 1.9% of applied dose).

*Abbreviations:* AA's, aromatic amines; BNA, beta-naphthylamine; CAS, chemical abstract service; CV, coefficient of variation; GC–MS, gas chromatography–mass spectrometry; HCl, hydrochloric acid; LOD, limit of detection; LogP, decadic logarithm of the octanol/water partition coefficient; NLGI, national lubricating grease institute; PBNA, *N*-phenyl-beta-naphthylamine; SD, standard deviation; SEM, standard error of the mean.

* Corresponding author.
*E-mail address:* Sonja.Kilo@fau.de (S. Kilo).

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*N*-Phenyl-beta-naphthylamine
Lubricant

The results clearly reveal that dermal penetration of both AA's depends strongly on the mode of application. Co-application and formulation alters the penetration of the AA's.



## 1. Introduction

Aromatic amines (AA's) are widely used in the production of rubber, dyes, pesticides or pharmaceuticals. Additionally, they are components of coal tar and pitch. Aromatic amines can be released into the environment by combustion of wood and fuel or from tobacco smoke. Since the majority of AA's is classified as carcinogenic for humans and/or animals (DFG, 2016) this group of substances is of concern for environment and human health and particularly for occupational health.

In the past, *N*-phenyl-beta-naphthylamine (PBNA; *N*-phenylnaphthalen-2-amine, CAS 135-88-6) was often implemented among others as antioxidant for lubricating oils and greases (Falbe and Regitz, 1991) in the rubber industry and other industrial workplaces. However, as a result of the manufacturing process PBNA was contaminated with beta-naphthylamine (BNA, naphthalen-2-amine, CAS 91-59-8) up to a concentration of 1000 mg/kg (DGUV, 2014). It has long been known that BNA can cause bladder cancer in humans which is the reason why it was substituted in the first place by the supposedly non-hazardous PBNA. However, based on data from animal studies, there are indications that PBNA too might be carcinogenic (DFG, 2016). Therefore the handling of contaminated lubricants may be associated with a considerable health risk for these workers.

Along with inhalation of vapors and dusts, skin contact is a common route of occupational exposure to a multitude of chemicals. For several AA's a substantial percutaneous penetration through animal (Baynes et al., 1996; Levillain et al., 1998) and human skin (Yourick and Bronaugh, 2000; Kenyon et al., 2004; Korinth et al., 2008) is confirmed. With 54% of the applied dose, a relevant penetration of BNA through human skin was demonstrated *ex vivo* (Lüersen et al., 2006). For PBNA qualitative and quantitative data on percutaneous penetration is scarce. In a diffusion cell study no penetration of PBNA through excised human skin was observed within an observation period of 24 h (Wellner et al., 2008). However, the study provides no information regarding the intradermal situation. Considering its high lipophilicity ($LogP_{OW}$ 4.38) it is possible that PBNA will be retained in skin as well as its passage through skin will be prolonged.

The use of an *ex vivo* model for the measurement of absorption of AA's in and trough human skin is essential since *in vivo* human studies cannot be implemented for carcinogenic substances. It is widely known that results obtained from animal studies are only to a limited extent transferable to humans (Jung and Maibach, 2015) due to differences in the epidermal barrier. Compared to this, the epidermal barrier function of freshly excised human skin *ex vivo* resembles *in vivo* conditions best.

The first aim of the present study was to establish an *ex vivo* penetration model for the study of dermal penetration of AA's. With this model, the dermal penetration (uptake into a certain layer or structure i.e. stratum corneum or deeper structures) and the resorption (uptake into systemic circulation or into its equivalent, the receptor fluid) characteristics of BNA and PBNA from a typical workplace lubricant as well as during and after direct, i.e. sole or mixed exposure, in and through freshly excised human skin were investigated.

## 2. Materials and methods

### 2.1. Test compounds and their physicochemical properties

Beta-naphthylamine and *N*-phenyl-beta-naphthylamine with a purity of 98 and 97%, respectively, were used for percutaneous penetration experiments. The aromatic amines (AA's) were purchased from Sigma Aldrich (Steinheim, Germany). With <0.0024%, PBNA used for experiments offered the lowest BNA impurity among different commercial suppliers (Weiss et al., 2013). The physicochemical properties of test compounds are shown in Table 1. Data were obtained from the PhysPro© Database of Syracuse Research Corporation (SRC, Syracuse, NY, USA; http://www.syrres.com).

### 2.2. Preparation of skin membranes

Freshly excised human skin was used for percutaneous penetration experiments. Skin from the abdominal area of 2 female donors (44 and 73 years) was obtained anonymously from a local clinic immediately following abdominoplasty. The study was performed according to ethical guidelines of our university and patients gave written informed consent. After removing the subcutaneous fat tissue the skin was prepared to a thickness of ~0.9 mm using a scalpel. Skin integrity was assessed visually prior to mounting the skin on diffusion cells by scanning the surface for macroscopic anomalies of skin structure, e.g., scars, striae or holes. Following equilibration for about 30 min, skin surface temperature was measured by a digital precision thermometer (GMH 1160 with GOF 500 universal probe, type K; Greisinger electronic GmbH, Regenstauf, Germany).

### 2.3. Preparation of test solutions and dermal application of lubricant

Industrial lubricant (calcium soap grease, NLGI consistency number 1), spiked with BNA and PBNA at a dose of 20 mg/kg (0.002%) and 10 g/kg (1%), respectively, was produced by and purchased from Zeller + Gmelin GmbH & Co. KG (Eislingen, Germany). The viscous lubricant was filled in a 1 ml syringe (Omnifix® – F 1 ml; B. Braun, Melsungen, Germany) and centrifuged (1 min, 1500 rpm to remove air bubbles). An amount of 0.035 g of the lubricant was squeezed on the skin fixed in diffusion cells (~40 µl), and distributed evenly with an embossing tool (Ø 5 mm). In preliminary tests (n = 9) the weight difference of the applicator before and after spreading the lubricant on the skin surface was measured with an analytical precision balance (XA105DU; Mettler Toledo®, Giessen, Germany) and the final concentration of lubricant applied to skin calculated (mean ± SEM: 0.026 ± 0.0005 g/0.64 $cm^2$).

According to these results, solutions of BNA (4.1 µg/l) and PBNA (2023.4 µg/l) in hexane were prepared for single, as well as for mixed exposure.

### 2.4. Percutaneous penetration experiments

Percutaneous penetration of BNA and PBNA was investigated using static PermeGear® diffusion cells (flat flange joint vertical system; 9 mm orifice; exposure area 0.64 $cm^2$; receptor chamber