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New experimental data on the human dermal absorption of Simazine and Carbendazim help to refine the assessment of human exposure

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HIGHLIGHTS

- Absorption kinetics of Simazine and Carbendazim were measured for the first time.
- Human skin was used to experimentally determine the permeability coefficient.
- Risks due to dermal exposure to polluted water were assessed probabilistically.
- Two exposure scenarios were considered and no increased risks were found out.
- New refined tool to assess the risks of dermal exposure to pollutants is presented.

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ABSTRACT

Due to their widespread usage, people are exposed to pesticides on a daily basis. Although these compounds may have adverse effects on their health, there is a gap in the data and the methodology needed to reliably quantify the risks of non-occupational human dermal exposure to pesticides.

We used Franz cells and human skin in order to measure the dermal absorption kinetics (steady-state flux, lag time and permeability coefficient) of Carbendazim and Simazine. These parameters were then used to refine the dermal exposure model and a probabilistic simulation was used to quantify risks resulting from exposure to pesticide-polluted waters.

The experimentally derived permeability coefficient was 0.0034 cm h^{-1} for Carbendazim and 0.0047 cm h^{-1} for Simazine. Two scenarios (varying exposure duration and concentration, i.e. environmentally relevant and maximum solubility) were used to quantify the human health risks (hazard quotients) for Carbendazim and Simazine. While no risks were determined in the case of either scenario, the permeability coefficient, which is concentration independent and donor, formulation, compound and membrane specific, may be used in other scenarios and exposure models to quantify more precisely the dermally absorbed dose during exposure to polluted water.

To the best of our knowledge, the dermal absorption kinetics parameters defined here are being published for the first time. The usage of experimental permeability parameters in combination with probabilistic risk assessment thus provides a new tool for quantifying the risks of human dermal exposure to pesticides.

1. Introduction

Today, many pesticides are used in the environment to protect crops against pests and to help increase agricultural production. Their use poses risks to both surface and ground water (Rojas et al., 2015). Pesticides are emitted into the environment by diffusive pathways and direct losses (De Wilde et al., 2009). While these compounds have toxic effects on target organisms, they also influence non-target organisms – including humans. Consequently, the use of personal protective equipment (PPE) is recommended for workers and operators who are occupationally exposed to them, in order to avoid or minimize risks resulting from such exposure. However, since other population groups, such as residents and bystanders, may also be exposed to these compounds due to environmental pollution, a greater degree of attention should be

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dedicated to human risk assessment associated with non-occupational exposure. Moreover, many people are vulnerable to non-agricultural sources of pesticide exposure and more data and research are thus needed to quantify such exposure (McKinlay et al., 2008). Nevertheless, there is a lack of experimental data on pharmacokinetics, including the transdermal penetration parameters of pesticides, and dermal non-occupational exposure thus cannot be quantified. Dermal exposure to pesticides may be an important source of exposure. Further actions focusing on clarifying the contribution of the dermal route to the total uptake of pesticides is needed (Beamer et al., 2009) and an experimental approach in assessing human exposure is crucial (Kefeni and Okonkwo, 2014).

This study focuses on two compounds: Simazine and Carbendazim. These two compounds were selected from among other pesticides according to their parameters (e.g. molecular weight, octanol–water partitioning coefficient and acceptable daily intake values), which influence their dermal absorption rate and thus risk of human exposure. In addition, no experimental data on their dermal absorption parameters has been published to date. As a result, it has been impossible to quantify their dermal exposure and the risks these compounds pose to humans.

Carbendazim is a fungicide from the benzimidazole group used to control plant diseases by inhibiting mitosis and cell division. Carbendazim has been found to result in maternal and developmental toxicity in mammals (Sitarek, 2001; Minta et al., 2004). It exhibits toxicity by affecting the liver in rats (Muthuviveganandavel et al., 2008). Carbendazim has also been found to increase estrogen production by increasing aromatase activity (Morinaga et al., 2004), increase the androgen receptor mRNA in male rats (Hsu et al., 2011) and induce other endocrine disrupting effects (Maranghi et al., 2003; Rajeswary et al., 2007; Yu et al., 2009; Prashantkumar et al., 2012; Rama et al., 2014). It has also been found to cause hepatic and slight kidney dysfunctions in male goats (Waghe et al., 2013). Moreover, Carbendazim is a metabolite of Benomyl, another benzimidazole pesticide. Carbendazim was previously used in the European Union; however, its inclusion on the list of active compounds approved in the EU (EC, 2009) expired in November 2014 (EC, 1995–2015). Nevertheless, plant protection products containing Carbendazim as an active compound are still available on the market and their use is approved until 2021, e.g. in the UK (HSE, 2015). Carbendazim can persist in the environment due to its benzimidazolic ring (Hernandez et al., 1996; Pourreza et al., 2015).

Simazine is a herbicide from the triazine group used to control broad-leaved weeds and annual grasses on deep-rooted crops, as well as on non-crop areas such as farm ponds and fish hatcheries (University of Hertfordshire, 2013). Its mode of action consists of photosynthesis inhibition. In mammals, Simazine is suspected of endocrine disruptive effects and there is evidence that it may be capable of inducing aromatase activity and thus increase estrogen production (Sanderson et al., 2000). It also exhibited developmental toxicity in female offspring mice by disturbing cellular apoptosis and proliferation during exposure *in utero* (Park et al., 2014). Simazine is on the list of 33 priority substances of the Directive 2008/105/EC (EC, 2008) amended by Directive 2013/39/EU (EU, 2013). This list was established in order to provide measures designed to reduce water pollution by such compounds by the Water Framework Directive (EC, 2000), which aims to reduce the negative impact of water pollution on the environment and on humans. Although the use of Simazine in the European Union was banned in 2004 (EC, 2004) due to environmental concerns (EC, 2003), Simazine and other triazine herbicides are still detected in the environment due to their long retention time in the soil and aquifers. Thus, due to leaching processes, levels of Simazine may be detected in the environment even years after their prohibition (Lorente et al., 2015). Also, Simazine is still used in a number of countries such as Australia (https://portal.apvma.gov.au).

In view of the above, measuring human exposure to pesticides such as Simazine and Carbendazim is necessary. Human skin *ex vivo* is currently the best available model for measuring the human transdermal penetration of chemical compounds (Barbero and Frasch, 2009). In a system of diffusion Franz cells, it presents an optimal tool for assessing human dermal exposure to Simazine and Carbendazim. The dermal absorption kinetics (steady state flux, permeability coefficient, lag time) of these pesticides measured by this system can serve as inputs for pharmacokinetic models; e.g. the US EPA exposure model allows for the calculation of dermally absorbed doses during defined common exposure scenarios. Refining this model by using experimentally derived pharmacokinetic parameters and probabilistic modeling can yield more realistic results when quantifying human non-occupational exposure to Carbendazim and Simazine in polluted water.

This study thus uses the above mentioned approach to quantify non-occupational dermal exposure to polluted water and thus assess the risks posed to humans by Simazine and Carbendazim and suggest measures for alleviating potential human health risks.

2. Materials and methods

2.1. Chemicals

All chemicals, Carbendazim (CAS number: 10605-21-7) analytical standard with 99.2% purity, Simazine (CAS number: 122-34-9) analytical standard with 99.8% purity, 0.01 M phosphate-buffered saline (PBS, containing 0.138 M NaCl; 0.0027 M KCl, pH = 7.4 at 25 °C), and bovine serum albumin (BSA, CAS number 9048-46-8) of ≥98% purity were purchased from Sigma-Aldrich. Milli-Q water was used to prepare all aqueous solutions.

2.2. Skin membranes source and preparation

The assay was performed following OECD guidelines (OECD, 2004a; OECD, 2004b) in accordance with Wellner et al. (2008) and Lademann et al. (2008).

For each compound, *ex vivo* human abdominal skin from 3 patients (males and female) was used. The patients were aged 27, 28 and 42 in the case of Carbendazim and 24, 27 and 46 in case of Simazine. The skin was obtained from plastic surgery with the informed consent of all patients.

After surgery, the skin was packed in a plastic bag, stored in the fridge and transported the laboratory at 4 °C. In the laboratory, the subcutaneous fat was removed with a scalpel. The hairs were thin and it was therefore not necessary to remove them. The skin was washed in tap and then distilled water, gently dried with a cotton tissue, cut into slices and packed in aluminum foil and a zip-lock bag. Finally, the skin was stored in a freezer at −20 °C for no longer than 6 months.

Immediately before the experiment, the skin was defrosted at ambient laboratory temperature and split-thickness membranes (350 μm) were prepared with an electric dermatome (Braun Acculan® 3Ti). The skin was cut into small pieces of uniform size and the integrity of the membrane was checked visually before the experiment. After the experiment, a post-study data analysis integrity evaluation was carried out (OECD, 2004a; Wellner et al., 2008; Stahl et al., 2012).

2.3. Dermal absorption experiment

An automatic MicroettePlus system (Hanson Research) with six