Toxicokinetics of captan and folpet biomarkers in dermally exposed volunteers.

Authors: Berthet A, Bouchard M, Vernez D

Journal: Journal of Applied Toxicology

Year: 2012

Volume: 3

Pages: 202-209

DOI: 10.1002/jat.1659
Toxicokinetics of captan and folpet biomarkers in dermally exposed volunteers

Aurélie Berthet,a,b Michèle Boucharda* and David Vernezb

a Département de santé environnementale et santé au travail, Chaire d’analyse et de gestion des risques toxicologiques and Institut de recherche en santé publique de l’Université de Montréal (IRSPUM), Faculté de Médecine, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec, Canada, H3C 3J7

b Institute for Work and Health, Rue du Bugnon 21, 1011 Lausanne, Switzerland

Correspondence to:
M. Bouchard, Département de Santé environnementale et santé au travail, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec, H3C 3J7, Canada.
E-mail: michele.bouchard@umontreal.ca
Telephone number: (514) 343-6111 ext 1640
Fax number: (514) 343-2200

Short title: Dermal kinetics of captan and folpet biomarkers
ABSTRACT: To better assess biomonitoring data in workers exposed to captan and folpet, the kinetics of ring metabolites (tetrahydrophthalimide (THPI), phthalimide (PI) and phthalic acid) were determined in urine and plasma of dermally exposed volunteers. A 10 mg kg\(^{-1}\) dose of each fungicide was applied on 80 cm\(^2\) of the forearm and left without occlusion or washing for 24 h. Blood samples were withdrawn at fixed time periods over the 72 h following application and complete urine voids were collected over 96 h post-dosing, for metabolite analysis. In the hours following treatment, a progressive increase in plasma levels of THPI and PI was observed, with peak levels being reached at 24 h for THPI and 10 h for PI. The ensuing elimination phase appeared monophasic with a mean elimination half-life (t\(_{1/2}\)) of 24.7 and 29.7 h for THPI and PI, respectively. In urine, time courses PI and phthalic acid excretion rate rapidly evolved in parallel, and a mean elimination t\(_{1/2}\) of 28.8 and 29.6 h, respectively, was calculated from these curves. THPI was eliminated slightly faster, with a mean t\(_{1/2}\) of 18.7 h. Over the 96-h period post-application, metabolites were almost completely excreted, and on average 0.02% of captan dose was recovered in urine as THPI while 1.8% of the folpet dose was excreted as phthalic acid and 0.002% as PI, suggesting a low dermal absorption fraction for both fungicides. This study showed the potential use of THPI, PI and phthalic acid as key biomarkers of exposure to captan and folpet.

Keywords: toxicokinetics; captan; folpet; tetrahydrophthalimide; phthalimide; phthalic acid; dermal exposure; human; biomarker

Table of Contents – Short abstract

To better assess biomonitoring data in workers exposed to captan and folpet, the kinetics of ring metabolites were determined in urine and plasma of dermally exposed volunteers. Each fungicide was applied on 80 cm\(^2\) of the forearm for 24 h. Blood samples and complete urine voids were collected respectively over 72 h and 96 h following application. Overall, the studied metabolites appeared as key biomarkers of exposure to captan and folpet despite the low dermal absorption fraction.
INTRODUCTION

The skin is in many instances the primary route of pesticide exposure for agricultural workers, as demonstrated by several studies (Ritcey et al., 1987; Fenske, 1990; Woollen, 1993; Ngo et al., 2010). It was estimated by a number of authors to account for 90% of exposure whatever the activity performed (i.e. preparation of mixture, spraying, material washing, harvesting) (Fenske, 1990; Vermeulen et al., 2002; Ngo et al., 2010). However, numerous factors influence dermal penetration of compounds and affect exposure measurements: physicochemical properties of compounds (solubility, chemical structure, partition coefficient), environmental conditions (humidity, temperature, wind), vehicle used to apply pesticides (physicochemical properties, solubility, pH, concentration), skin properties and cutaneous metabolism (skin condition, skin hydration, type, thickness, enzyme activities, wounds), shunt diffusion, anatomical site of exposure (skin thickness, hair follicle size and density), inter-individual variability (age, gender, race), dose, duration and occlusion (Lotte et al., 1993; Kromhout and Vermeulen, 2001; Tsai et al., 2001; Mangelsdorf et al., 2006). Therefore, biological monitoring becomes a reliable means of estimating dermal exposure in humans as underlined by several authors (de Cock et al., 1995; Ngo et al., 2010; van Welie et al., 1991). Nonetheless, to be accurate, biomonitoring data need to be supported by adequate kinetic studies in human volunteers (Wester and Maibach, 1983; Woollen, 1993; Ngo et al., 2010).

Captan and folpet are considered as two commonly used fungicides in various crops of many countries (Guo et al., 1996; Fenske et al., 1998; Tieleman et al., 1999; Carden et al., 2005; Baldi et al., 2006; Juras et al., 2007; Greenburg et al., 2008). However, only a limited number of studies are reported in the literature that use biomonitoring to assess captan exposure in workers (Hansen et al., 1978; Mcjilton et al., 1983; Winterlin et al., 1984; Winterlin et al., 1986; Verberk et al., 1990; Krieger and Thongsinthusak, 1993; Lavy et al., 1993; de Cock et al., 1995, 1998; Krieger and Dinoff, 2000; Hines et al., 2008). Moreover, to our knowledge, no reports are available that describe biomonitoring of folpet. As for kinetic studies in human volunteers, one is described in the literature following an oral exposure to captan (Krieger and Thongsinthusak, 1993) while such data is lacking for folpet.

On the other hand, the metabolism of both fungicides is well documented in in vivo studies in animals usually exposed to the radiolabelled compounds and in in vitro studies. According to these data, the first step in the metabolism of captan is its breakdown at the N-S link when it is in contact with thiol groups (e.g. glutation, cysteine, proteins, etc.), and the reaction is enhanced in alkaline medium (Wolfe et al., 1976). From this nonenzymatic process a tetrahydroptalimide (4,5-cyclohexene-1,2-dicarboximide; THPI) and a thio carbonyl chloride are formed, the latter being a derivative of the trichloromethylthio group. This thio carbonyl chloride further reacts with thiols to form thiophosgene, a transient metabolite, which readily reacts with cysteine or glutathione hence leading to the formation of thiazolidine-2-thione-4-carboxylic acid (TTCA); two other metabolites can also be formed, the dithiobis (or methanesulfonic acid) and its disulphide monoxide derivative, arising from the reaction with sulphite ions (DeBaun et al., 1974). From animal studies and two occupational studies carried out by Winterlin et al. (1984, 1986) in strawberry pickers and in grape field workers, THPI was a suggested as possible biomarker of captan exposure by the Environmental Protection Agency (US EPA) (1975).
Similar to captan initial metabolism step, folpet is also broken down at the N-S link when it is in contact with thiol groups; this leads to the formation of a phthalimide (PI) and a thiocarbonyl chloride, but unlike for captan, the reaction is enhanced in acid conditions (Gordon et al., 2001; Gordon, 2010). From the thiocarbonyl chloride, the same metabolic reactions as captan occur and the same thiol metabolites are formed. On the other hand, the ring metabolite of folpet, PI, is rapidly hydrolyzed to phthalamic acid mainly but also to phthalic acid, according to animal studies (Gordon et al., 2001; Zainal and Que Hee, 2003; Canal-Raffin et al., 2008; Gordon, 2010). For the biological monitoring of exposed individuals, no biomarker was proposed for this fungicide since no human study was reported.

Overall, although some metabolism data are available from animal studies for these two fungicides, there is a paucity of kinetic data in humans following dermal exposure. These human kinetic data are especially needed given the well known interspecies differences in dermal absorption (Feldmann and Maibach, 1974; Wester and Maibach, 1983; Kao and Carver, 1990; Fiserova-Bergerova, 1993; Fenske et al., 1998, Poet and McDougal, 2002). In addition, the dermal route is in many circumstances the main route of exposure to pesticides in workers.

The aim to this study was thus to determine the kinetics of captan and folpet ring metabolites in accessible biological matrices of dermally exposed volunteers to better assess biomonitoring data in workers. It also aimed to compare the toxicokinetics of captan and folpet metabolites, given the related chemical structure of the parent compound and initial breakdown metabolites.
MATERIALS AND METHODS

Study design

The study design was similar to the one previously described for volunteers orally exposed to the same fungicides and reported in Berthet et al. (2011a). Thus, a controlled kinetic time course study in accessible biological matrices of healthy subjects was conducted following a dermal exposure to captan and folpet. Captan and folpet metabolites were measured in urine and blood samples collected prior to treatment to obtain pre-test values, and then at predetermined time points post-dosing (amounting to a total of 9 samples for plasma and 11 samples for urine).

The experimental protocol and consent forms were approved by the Permanent Ethics Committee for Clinical Research of the Faculty of Biology and Medicine of the University of Lausanne and the Research Ethics Committee of the Faculty of Medicine of the University of Montreal. All the participants gave their written consent, and were informed of the risks of participating and their right to withdraw from the study at anytime. As suggested by the Ethics Committee who considered the study as very restrictive, the participants received a monetary compensation for their time and any inconvenience caused.

Subjects studied

Participants were recruited on a voluntary basis among the medical students of the University of Lausanne, Switzerland. The volunteers were nine male students aged from 20 to 30 years old, weighing 60 to 85 kg and measuring 169 to 184 cm. They were healthy, non-smokers and did not take medication or drugs. They underwent a medical examination by an occupational physician prior to enrolment. They had never been exposed to captan or folpet, except an acute oral dose of 1 mg kg⁻¹ two weeks before, and perhaps through their diet. During the study period and the two-days prior to dosing, they were asked not to eat fruits and vegetables in order to limit ingestion of contaminated food. They were also requested to avoid consumption of alcohol during this period, since it is known to affect the metabolism of some chemical compounds.

Dosing and sampling

The experimental dosing and sampling was also conducted at the Institute for Work and Health of Lausanne, Switzerland. The morning of study onset, each participant was asked to collect his complete first morning urine void, to obtain pre-test values; they then spent the first day of the study in a room at the Institute for dosing and the first 12-h sampling. On the following 3-day sampling period, participants visited the Institute for their daily morning blood sampling and handing-in urine collections.

A dose of captan or folpet equivalent to 10 mg kg⁻¹ body wt was applied on 80 cm² of one of the forearm of each volunteer (n = 5 for captan and n = 4 for folpet). Fungicides used to prepare the exposure dose of volunteers were Captan PESTANAL® (assay (HPLC) area 99.1 to 99.4%) and Folpet PESTANAL® (assay (HPLC) area 99.5 to 99.9%). Both were purchased from Fluka – Sigma-Aldrich (Buchs, St Gallen, Switzerland). The single dose was weighted in a labelled glass
container and adjusted to the weight of each volunteer. The fungicide dose was dissolved in 30 mL of acetone, mixed and applied on the skin. To ensure that the dose was applied on 80 cm², a 20 x 4 cm cardboard frame was fixed on the forearm of each volunteer to delimit the area. To avoid injuring the participant, a plastic pipette was used to apply the dose. The participants washed their arm only 24 h following application. The exposed area was not occluded to avoid influencing absorption (Feldmann and Maibach, 1974; Wester and Maibach, 1983; Curdy et al., 2004; Carden et al., 2005), except some of the participants wore long sleeve shirts since the study was performed during the winter season.

Complete micturitions were then collected at pre-determined times over the 96–h period post-dosing, that is at around 0, 3, 6, 9, 12, 24, 36, 48, 60, 72, 84 and 96 h post-dosing. Each timed-void was collected in separate polypropylene Nalgene® bottles of 1 l. To assist participants, they received a schedule specifying the date and the required time of urine collection. They then only had to complete the sheet with the actual time of collection and to indicate whether or not there were any urine losses. They also had to identify their plastic bottles with distinct pre-printed labels indicating the code, and mark the date and time of urine collection.

Once collected, urine samples were kept in the refrigerator prior to measurement of total urine volume per void. To allow repeated analysis while avoiding possible degradation due to freezing and defreezing of samples, each urine collection then was aliquoted in 4 labelled tubes of 15 ml and one bottle of 120 ml before storage at -20°C until analysis of captan or folpet metabolites.

Blood samples of 15 ml were also collected at specific times, that is at t = 0, 2, 4, 6, 8, 10, 24, 48 and 72 h post-dosing. To facilitate collection, a catheter was installed by a nurse prior to dosing along with a drip system of physiological saline the first day of sampling. Blood was withdrawn by the nurse into vacutainers pre-labelled with a code, the date and time of sampling. Immediately after collection, blood samples were centrifuged to precipitate red blood cells and isolate plasma. The plasma samples were then split into 3 labelled aliquots and stored at -20°C until analysis of captan and folpet metabolites.

During the study period, volunteers were also asked to fill a questionnaire to document personal information (weight, height), life habits (i.e. physical activities, smoking), medication intake (including ibuprofen), alcohol consumption during the 3 days prior to treatment, consumption of fruits, vegetables and cereals during the 4 days prior to treatment and possible dosing-related symptoms.

Chemicals and reagents

Reference standards (>99% purity) were obtained from Sigma-Aldrich (Buchs, St Gallen, Switzerland), except for deuterated cis-1,2,3,6-tetrahydrophthalimide (THPI-d) (99% purity), which was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). HPLC grade acetonitrile, methanol, ethyl acetate and dichloromethane were also obtained from Sigma-Aldrich (Buchs, St Gallen, Switzerland), along with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), the reagent used to derivatize the phthalic acid molecule. HCl and ammonium sulphate were purchased from Merck (Zug, Switzerland). Water was purified using a TKA GenPure water treatment system obtained from TKA Wasseraufbereitungssysteme GmbH (Niederelbert, Germany).
Analysis of THPI and PI in plasma and urine

A liquid chromatography - atmospheric pressure chemical ionization-tandem mass spectrometry (LC/APCI-MS/MS) method was developed to analyze THPI and PI in urine and plasma, and is described elsewhere (Berthet et al., 2011b). Briefly, THPI and PI were isolated by solid phase extraction (SPE) (in the case of plasma, following a denaturation of proteins), eluted in dichloromethane and analyzed using a Varian Model 212-LC Binary Gradient LC system (Les Ulis, France) connected to a Prostar model 410 autosampler (Varian, Les Ulis, France) and coupled to a Model 1200 L quadrupole MS (Varian, Les Ulis, France) operating in APCI mode. The APCI interface was operated in negative ion mode. The compounds were separated using a C18 Zorbax Eclipse Plus column (4.6 x 150 mm, 3.5 µm) from Agilent (Morges, Switzerland). The mobile phase consisted of: eluent A composed of 90% water and 10% acetonitrile (9:1), and eluent B of 10% water – 90% acetonitrile (1:9). For THPI analysis, 10 µl of the sample were injected and elution was performed in 26 min using a solvent gradient at a flow rate of 0.8 ml min⁻¹, and for PI analysis, 10 µl of the sample were also injected and elution was performed in 33 min in isocratic mode at a flow rate of 0.8 ml min⁻¹. The fragments analyzed were \( m/z \) 149.4/95.6 for THPI with a collision energy (CE) of 19.5 V, \( m/z \) 156.1/95.6 for the internal standard THPI-d (CE of 22.5 V) and \( m/z \) 145.8 for PI (no fragmentation). The analytical limit of detection for THPI was 3.82 nmol l⁻¹ of urine and 9.76 nmol l⁻¹ of plasma and, for PI, 7.72 nmol l⁻¹ of urine and 14.8 nmol l⁻¹ of plasma. The quantification of THPI or PI was obtained from standard calibration curves prepared in urine or plasma adjusted by the THPI-d internal standard surface area.

Analysis of phthalic acid in urine

A gas chromatography mass spectrometry (GC-MS) method was developed for the analysis of phthalic acid in urine, as previously described (Berthet et al., 2011c). In short, urine samples were subjected to an acid hydrolysis prior to liquid-liquid extraction with ethyl acetate and derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Analysis was then performed using an Agilent Mass Selective Detector (MSD) G1098A (Agilent Technologies Inc, Waldbronn, Germany) coupled with a HP 5973 mass spectrometer (Agilent Technologies Inc, Waldbronn, Germany). Separation of the analytes was performed using a 60 m CP-SIL 8 CB column (1 µm film thickness, 250 µm I.D.) (Varian, Les Ulis, France). The initial column temperature was 200°C for 3 min, then it was increased to 260°C at 30°C min⁻¹, held for 11 min, and finally increased to 280°C at 35°C min⁻¹ and held for 4 min. For the analysis, 2 µl were injected using a 5 ml min⁻¹ split. The ions monitored were trimethylsilyl phthalic acid (TMS phthalic acid) with \( m/z \) 295 and the internal standard TMS methylhippuric acid with \( m/z \) 220. The quantification was obtained from standard calibration curves of phthalic acid prepared in urine and adjusted by the methylhippuric acid internal standard surface area. The analytical limit of detection was 0.6 µmol l⁻¹ urine.

Calculations

The molar fraction of captan or folpet administrated dose recovered in urine as THPI, PI or phthalic acid was calculated from the following equation:
where \( Q_{urinary metabolite} \) corresponds to total amounts of THPI, PI or phthalic acid in urine over the 96-h urine collection period (mg), \( MW_{parent compound} \) is the molecular weight of captan or folpet, \( MW_{metabolite} \) is the molecular weight of THPI, PI or phthalic acid and \( Dose_{parent compound} \) is the orally administered dose of captan or folpet (mg).

**Toxicokinetic analysis**

To determine elimination rate constants of THPI and PI in plasma and in urine, we considered that, following peak levels, elimination was monophasic and a first-order reaction. The elimination rate constant \((k)\) was thus calculated from the slope of the linear terminal phase of the plasma or urine time profile. The apparent elimination half-life \((t_{1/2})\) was then defined using the equation \( t_{1/2} = 0.693 k^{-1} \) (Hayes, 2008).

From plasma concentration \((C)\) - time profile, we also calculated the area under the concentration-time curve \((AUC)\), the area under the first moment of concentration-time curve \((AUMC)\), the mean residence time \((MRT)\), the plasma clearance \((CL)\) and the apparent volume of distribution \((V_d)\). Equations used to calculate these parameters are:

\[
AUC = \frac{1}{2} \sum_{i} (t_i - t_{i+1}) [C(t_i) + C(t_{i+1})]
\]

\[
AUMC = \frac{1}{2} \sum_{i} (t_i - t_{i+1}) [t_i C(t_i) + t_{i+1} C(t_{i+1})]
\]

\[
MRT = \frac{AUMC}{AUC}
\]

\[
CL = \frac{Absorbed dose}{AUC}
\]

\[
V_d = \frac{CL}{k} \quad \text{where } k \text{ is the overall elimination rate from blood}
\]
RESULTS

Time courses of THPI and PI in plasma

The time courses of THPI and PI in plasma of volunteers over the 72-h period following an application of captan or folpet (10 mg kg\(^{-1}\) body wt) are presented in Fig. 1. In control samples taken prior to captan or folpet ingestion (\(t_0\)), concentrations of THPI were below the analytical limit of detection and those of PI were very low, in the order of 3.9 nmol l\(^{-1}\). Following application, a progressive increase in plasma levels of THPI and PI was observed, with peak levels being reached 24 h post dosing for THPI and 10 h post-treatment for PI. Subsequent elimination phase of THPI and PI from plasma over the 24- to 72-h period post-dosing appeared monophasic with an apparent elimination half-life of 24.7 and 29.7 h, respectively (Table 1). From the THPI and PI time profiles in plasma, various toxicokinetic parameters were calculated and are presented in Table 2. These show that following dermal application, THPI had a greater bioavailability and slightly faster clearance rate than PI; on the other hand, they had similar, relatively small, volume of distribution (\(V_d\)).

Time courses of THPI, PI and phthalic acid in urine

Figure 2 presents the time courses of THPI, PI and phthalic acid excretion rate in the urine of volunteers over a 96-h period following application of 10 mg kg\(^{-1}\) body wt of captan or folpet. As expected, these profiles evolved in unison with the blood profiles (see Figure 1 for comparison). It is readily observed from Fig. 2 that, following peak excretion, elimination rate time courses of PI and phthalic acid were parallel, with mean apparent elimination half-lives of 28.8 and 29.6 h, respectively, calculated for the 36- to 96-h period post-dosing (Table 1). THPI elimination was slightly faster with an apparent elimination half-life of 18.7 h calculated for the same period (Table 1).

Figure 3 displays the corresponding cumulative urinary excretion time courses of the three metabolites over the 96-h collection period post-dosing. From these data, it was calculated that 1.8% of the applied dose of folpet was recovered in urine as phthalic acid as compared to only 0.002% for PI. This indicates that phthalic acid was present in urine in 1 000-fold higher amounts than PI despite the fact that they had similar urinary time-profiles and thus elimination half-lives. By comparison, the percentage of applied captan dose recovered in urine as THPI was 0.02%, which is 10-fold higher than the percentage of applied folpet dose found as PI in urine.
DISCUSSION

Kinetics of biomarkers of exposure to captan and folpet in dermally exposed volunteers

The present study provided novel data on the toxicokinetics of key biomarkers of exposure to captan and folpet following dermal exposure in humans. Such dermal kinetic data were lacking for both fungicides, not only in humans but also in animals. The studied biomarkers were found to be rapidly formed and eliminated from the body, with elimination half-lives following dermal application in the order of 20-25 h for THPI and 30 h for folpet ring-metabolites. No built up in the body was apparent; elimination from the body was complete 96 h post-treatment. The small percentage of dose recovered in urine as the studied biomarkers also suggests a low dermal absorption fraction of both captan and folpet.

Although dermal kinetic data on these specific biomarkers were not available in the literature for comparison purpose, the dermal absorption, tissue distribution and excretion of total radioactivity has been documented in animals dermally exposed to labelled captan or folpet (Grissom *et al*., 1985; Shah *et al*., 1987; Fisher *et al*., 1992). These animal data are in accordance with current results, showing a rapid elimination of labelled dose following dermal exposure and a low dermal absorption fraction. In particular, in one of the few published dermal kinetic studies of captan in animals, Fisher *et al.* (1992) observed that, on average, 82% of a dermally applied dose of 14C-ring-captan (286 nmol cm-2) were recovered in the analyzed tissues and fluids (kidneys, liver, total skin, carcass, urine, feces and blood) of aging Fischer 344 female rats (33 and 82 days) over the 0-120 h collection period. After 120 h, on average, 10.8 and 11.5% of the applied dose had penetrated young and adult skin, respectively, and 9% of dose in young rats and 10% of dose in adults were recovered as total radiactivity in urine. Similar to published oral experiments in rats exposed to ring-labelled captan, this implied that most of the absorbed dose was excreted in urine while very little was excreted in feces.

Similarly, in one of the only dermal penetration study on folpet in animals, Shah *et al.* (1987) estimated a low dermal absorption fraction, as assessed from analysis of radioactivity in skin and carcasses at 72 h post-application of a low, medium and high dose of 14C-trichloromethyl labelled folpet in Fisher 344 rats as well as in urine and feces collected over the 72-h period post-dosing. On average 12% of the low dose of folpet were calculated to be absorbed through the skin during that time (application of 0.1 µmol cm-2 on 2.8 and 5.6 cm² of young and adult rats, respectively) as compared to only 3 and 1 % for the two higher doses (application of 0.5 and 2.7 µmol cm-2, respectively).

The study of Krieger and Thongsinthusak (1993) is the only available published time course study of specific THPI biomarker in the urine of volunteers exposed under controlled conditions. In the latter study, two volunteers were orally exposed to 0.1 and 1 mg kg⁻¹ captan and it was observed that THPI represented 1 to 3% of the administered dose. In a preliminary attempt, captan was also applied to the hands, forearms and inguinal region of the volunteers (15 mg on 40 µg cm⁻² region of the skin of volunteers weighing 150 and 84 kg) but the dose was too low to allow quantification of THPI in urine. Nonetheless, they estimated a captan dermal absorption of 0.3% per day for humans.
Furthermore, from the observed kinetic data in the current study, some essential biological determinants can be identified. In particular, the dermal kinetics of the two studied biomarkers of exposure to folpet, PI and phthalic acid, exhibited similar time profiles, indicating that they were governed by the same essential biological processes. However, phthalic acid was found to be present in much higher amounts than PI in urine, in line with the substantial metabolism of PI into acids documented by some authors following oral or intraperitoneal exposure (Ackermann et al., 1978; Chasseaud et al., 1991; Canal-Raffin et al., 2008; Gordon, 2010). In particular, Canal-Raffin et al. (2008) reported an elimination half-life of on average 2.5 h for PI in plasma following a single intraperitoneal folpet dose of 10 mg kg\(^{-1}\) in Wistar rats. Ackermann et al. (1978) also estimated a PI half-life of 2 h in rat fetuses following an oral administration of 2.5 mg kg\(^{-1}\) of \(^{15}\)N-phthalimide to pregnant Wistar-strain albino rats and observed a fast metabolism of PI into phthalamic acid. This latter metabolite is then transformed to phthalic acid, the final ring-metabolite of folpet (Williams and Blanchfield, 1974).

By comparison, current results suggest that THPI is a minor metabolite of captan, since urinary amounts were found to represent only a very small percentage (0.02%) of the dermal dose in volunteers. This is corroborated by oral mass-balance data in animals following radiolabelled doses, with specific identification of metabolites in urine, showing in particular the presence of 3-hydroxy-4,5-cyclohexene-1,2-dicarboximide (3-OH-THPI) derivative as a more important metabolite (Lappin and Havell, 1990; Gordon, 2010).

**Comparison of the kinetics of captan and folpet ring metabolites in dermally exposed volunteers**

This study also allowed comparing the kinetics of captan and folpet ring metabolites in dermally exposed volunteers. Although these biomarkers are rapidly eliminated from the body following dermal exposure with apparent negligible tissue accumulation, differences in the dermal kinetics of THPI ring-metabolite of captan as compared to that of PI and phthalic acid ring-metabolites of folpet were observed. THPI was eliminated slightly faster than folpet ring-metabolites. Dermal absorption fraction also appeared more important for folpet than captan when comparing amounts of metabolites recovered overall in urine (1.8% of dermal folpet dose recovered as phthalic acid in urine as compared to 0.02% of dermal captan dose found as THPI in urine with reference to 25 and 3.5% respectively after oral administration).

**Comparison of the dermal and oral kinetics of ring-metabolites of captan and folpet**

When compared to oral kinetics, the dermal kinetics of captan and folpet ring-metabolites in humans exhibited marked differences. Indeed, the plasma time profile of THPI observed in the current study following dermal application in volunteers together with calculated toxicokinetic parameters can be compared to the ones described in Berthet et al. (2011a) following an oral administration in the same volunteers. The apparent elimination half-life of THPI calculated from the terminal elimination phase along with the plasma clearance indicate a somewhat slower elimination of THPI from plasma following dermal application than after ingestion (average t\(_{1/2}\) of 24.7 and 15.7 h, respectively; mean CL of 0.24 and 0.18 l h\(^{-1}\), respectively). THPI was also found to have a 10-fold lower area under the curve following dermal application than oral exposure (average AUC of 137.3 and 1650 (nmol x h l\(^{-1}\)) kg\(^{-1}\)), respectively. As compared to ingestion,
dermal exposure is thus associated with a lower absorption fraction and slower absorption rate, the latter influencing the elimination rate of THPI from plasma.

Similarly, the current time courses of PI in plasma of volunteers and associated toxicokinetic parameters can also be compared to the oral time courses in the same volunteers described in Berthet et al. (2011a). The apparent elimination half-life and plasma clearance rate of PI were similar for both routes of exposure, showing a negligible effect of the dermal absorption rate on elimination rate of PI from plasma (average $t_{1/2}$ of 29.7 and 31.5 h, respectively; mean CL of 0.13 and 0.09 l h$^{-1}$, respectively). On the other hand, PI was found to have a slightly higher area under the curve following dermal application than oral exposure (average AUC of 26.1 and 13.4 (nmol x h l$^{-1}$) kg$^{-1}$), respectively; this is compatible with a proportionally more extensive site-of-entry metabolism of PI into its derivatives following oral administration.

The urinary time course of THPI observed in this study following dermal application in volunteers can further be compared to the one reported in Berthet et al. (2011a) following an oral administration, again in the same volunteers. In accordance with the plasma profiles, the apparent elimination half-life of THPI calculated from urinary excretion rate time courses indicates a slightly slower elimination of THPI following dermal application than ingestion (average $t_{1/2}$ of 18.7 and 11.7 h, respectively). The percentage of captan dose recovered in urine as THPI was also 175 times lower following dermal exposure than after oral administration (on average 0.02 versus 3.5%), indicating a very low dermal absorption fraction.

Similarly, the dermal time courses of folpet metabolites in urine can be compared to the ones reported in Berthet et al. (2011a) following oral exposure in the same volunteers. In accordance with the plasma profiles, the apparent elimination half-lives of PI and phthalic acid were similar for both routes of exposure, showing a negligible effect of the dermal absorption rate on elimination rate of PI and phthalic acid (average $t_{1/2}$ of 28.8 and 27.3 h, respectively for PI, and 29.6 and 27.6 h for phthalic acid, respectively). On the other hand, the percentage of folpet dose recovered in urine as PI and phthalic acid was 10- and 14-fold lower, respectively, following dermal exposure than after oral administration (on average 0.002 versus 0.02% for PI and 1.8 versus 25% for phthalic acid), indicating a low dermal absorption fraction.

Animal studies also confirm a lower absorption fraction of captan and folpet following dermal exposure as compared to an oral exposure. Indeed, as mentioned previously, Fisher et al. (1992) estimated a dermal absorption fraction of on average 10.8 to 11.5% in Fisher rats based on the time course in skin and excreta over a 120-h period following a dermal dose of 286 nmol cm$^{-2}$. As for Grissom et al. (1985), they estimated a dermal penetration of 7.8% in Dublin ICR mice dermally exposed to 1 mg kg$^{-1}$ cm$^{-2}$ of $^{14}$C-captan, as assessed from analysis of radioactivity in skin, tissues and excreta (urine, feces and exhaled air) over a 24-h period post-dosing. Moreover, again as mentioned previously, on average 12, 3 and 1% of folpet were absorbed through the skin over a 72-h period following a dermal application of 0.1, 0.5 and 2.7 µmol cm$^{-2}$ of radiolabelled folpet in Fisher rats, respectively (Shah et al., 1987). By comparison, following oral exposure, Trivedi (1990) observed that about 75% of a 10 mg kg$^{-1}$ dose of $^{14}$C-ring labelled captan in male Sprague-Dawley rats were recovered in urine as $^{14}$C-equivalents over the 24-h period post-dosing as compared to 6.5% in feces. Similarly, following an oral administration of 10 mg kg$^{-1}$ of $^{14}$C-labelled folpet in rats, Chasseaud et al. (1991) also showed that 92% of the dose were recovered in urine as $^{14}$C equivalents as compared to 6% in feces.
Potential use of ring-metabolites of captan and folpet as biomarkers of exposure in workers on the basis of the current dermal kinetic data

The current dermal kinetic data in volunteers can serve to establish the potential use of ring-metabolites of captan and folpet as biomarkers of exposure in workers. Although THPI and PI in urine represented only a small fraction of the applied dose, their kinetics could easily be established in exposed volunteers. Given these data and the fact that THPI and PI are metabolites specific to captan and folpet, respectively, as well as the availability of simple and sensitive analytical methods for their detection, their use as bioindicators of exposure to captan and folpet should be further considered in worker biomonitoring studies. The measured phthalic acid is less specific since it is also a phthalate metabolite (Blount et al., 2000; Vermeulen et al., 2001; Kato et al., 2005; Lim et al., 2007, Silva et al., 2007). However, it is a major biomarker of folpet and its measurement could usefully serve in combination with that of PI to assess worker exposure.

It should be mentioned that the ring-metabolites are to be used as bioindicators of exposure and thus to reflect absorbed doses of captan or folpet. These biomarkers are not implicated in the pathways leading to toxicity. Eventual assessment of metabolites derived from the thiophosgene moiety (DeBaun et al., 1974) may help link biomarkers of exposure to potential health outcomes.

As compared to current results, some differences in the dermal absorption fraction and rate may be observed in the context of a dermal exposure in workers, given the use of acetone as a vehicle for dose administration in this study, which enhances permeability of the skin and thus absorption (Tsai et al., 2001). Some differences in the absorption could also occur in workers depending on the anatomical site exposed (Feldmann and Maibach, 1974). However, the forearm was selected in the present study as the application site, since hands and arms are the anatomical regions most exposed in workers (Mcjilton et al., 1983; Zweig et al., 1985; de Cock et al., 1998; Hughes et al., 2006; Ngo et al., 2010).

Overall, the present study provided new data of the dermal kinetics of key biomarkers of exposure to captan and folpet. It highlighted distinctions in the kinetics of captan and folpet phthalimide derivatives, but also route-to-route differences. However, THPI and PI appeared as convenient biomarkers to quantify captan or folpet exposure. As for phthalic acid, even if it is not a metabolite specific to folpet, it is a derivative of PI that can be used to corroborate results obtained for PI.
Acknowledgements

Authors wish to thank Dr. Michèle Berode and the technicians P. Stefan, M. Nobel and C. Kohler (Institute for Work and Health) for their help in organizing the study. We also thank Dr. Khanh Huynh (Institute for Work and Health) for his help with the LC/MS/APCI system. The study was funded by the ANSES (Agence Nationale de Sécurité Sanitaire de l’alimentation, de l’environnement et du Travail) and Aurélie Berthet received a Ph.D. scholarship from the Institut de recherche Robert-Sauvé en santé et sécurité du travail du Québec for this research.
REFERENCES


Trivedi S. Captan: Excretion mid tissue retension of a sngle pral dose (10 mg/kg) in the rat. ICI Central Toxicology Laboratory: Macclesfield. Unpublished report No CTL/P/2820.


Table 1. First-order elimination half-lives of THPI and PI in plasma and of THPI, PI and phthalic acid in urine following application of 10 mg kg\(^{-1}\) of captan or folpet on the forearm of volunteers.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Matrices</th>
<th>Mean first-order elimination half-life (h)(^{a})</th>
<th>Coefficient of determination (R(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>THPI</td>
<td>Plasma</td>
<td>24.7</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>18.7</td>
<td>0.86</td>
</tr>
<tr>
<td>PI</td>
<td>Plasma</td>
<td>29.7</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>28.8</td>
<td>0.90</td>
</tr>
<tr>
<td>Phthalic acid</td>
<td>Urine</td>
<td>29.6</td>
<td>0.86</td>
</tr>
</tbody>
</table>

\(^{a}\) The apparent elimination half-life (t\(_{1/2}\)) was calculated using the equation t\(_{1/2}\) = 0.693 \(k\)\(^{-1}\), where \(k\) is the elimination rate constant obtained from the slope of the linear terminal phase of the plasma profile (24-72 h) or urine excretion rate time course (36-96 h) for each metabolite.
Table 2. Toxicokinetic parameters for THPI and PI in plasma of volunteers following a dermal application of 10 mg kg\(^{-1}\) of captan or folpet on 80 cm\(^2\) of the forearm.

<table>
<thead>
<tr>
<th>Model parameters(^a)</th>
<th>First order toxicokinetic values</th>
<th>THPI Mean</th>
<th>THPI SD</th>
<th>Phthalimide Mean</th>
<th>Phthalimide SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n=5)</td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC [(nmol x h l(^{-1}) kg(^{-1})]</td>
<td>137.3</td>
<td>43.38</td>
<td>26.01</td>
<td>14.36</td>
<td></td>
</tr>
<tr>
<td>AUMC [(nmol x h(^2) l(^{-1})] kg(^{-1})]</td>
<td>4 772</td>
<td>1 929</td>
<td>532.2</td>
<td>152.5</td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>34.07</td>
<td>5.52</td>
<td>22.50</td>
<td>5.54</td>
<td></td>
</tr>
<tr>
<td>CL (l h(^{-1})]</td>
<td>0.24</td>
<td>0.07</td>
<td>0.13</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Vd (l)</td>
<td>7.44</td>
<td>2.14</td>
<td>6.07</td>
<td>2.57</td>
<td></td>
</tr>
</tbody>
</table>
Captions to figures

Figure 1. Time courses of THPI and PI in plasma of volunteers (expressed as nmol) over a 72-h period following a single application of 10 mg kg\(^{-1}\) of captan or folpet on 80 cm\(^2\) of the forearm. Each point represents mean and vertical bars are standard deviations.

Figure 2. Time courses of THPI, PI and phthalic acid excretion rate in urine (expressed as nmol/h) of volunteers over a 96-h period following a single application of 10 mg kg\(^{-1}\) of captan or folpet on 80 cm\(^2\) of the forearm. Each point represents mean and vertical bars are standard deviations.

Figure 3. Time courses of THPI, PI and phthalic acid cumulative excretion in urine (expressed as nmol) of volunteers over a 96-h period following a single application of 10 mg kg\(^{-1}\) of captan or folpet on 80 cm\(^2\) of the forearm. Each point represents mean and vertical bars are standard deviations.
Figure 1.
Figure 2.

![Graph showing urinary excretion rate vs. time after dose application](image-url)

- Phthalic acid
- THPI
- PI

Y-axis: Urinary excretion rate (nmol/h)
X-axis: Time after dose application (h)
Figure 3.