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Toxicokinetics of captan and folpet biomarkers in orally exposed volunteers

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Short title: Oral kinetics of captan and folpet biomarkers

ABSTRACT: The time courses of key biomarkers of exposure to captan and folpet was assessed in accessible biological matrices of orally exposed volunteers. Ten volunteers ingested 1 mg kg^{-1} body weight of captan or folpet. Blood samples were withdrawn at fixed time periods over the 72 h following ingestion and complete urine voids were collected over 96 h post-dosing. The tetrahydrophthalimide (THPI) metabolite of captan along with the phthalimide (PI) and phthalic acid metabolites of folpet were then quantified in these samples. Plasma levels of THPI and PI increased progressively after ingestion, reaching peak values ≈10 and 6 h post-dosing, respectively; subsequent elimination phase appeared monophasic with a mean elimination half-life ($t_{½}$) of 15.7 and 31.5 h, respectively. In urine, elimination rate time courses of PI and phthalic acid evolved in parallel, with respective $t_{½}$ of 27.3 and 27.6 h; relatively faster elimination was found for THPI, with mean $t_{½}$ of 11.7 h. However, phthalic acid was present in urine in 1 000-fold higher amounts than PI. In the 96-h period post-treatment, on average 25% of folpet dose was excreted in urine as phthalic acid as compared to only 0.02% as PI. Corresponding value for THPI was 3.5%. Overall, THPI and PI appear as interesting biomarkers of recent exposure, with relatively short half-lives; their sensitivity to assess exposure in field studies should be further verified. Although not a metabolite specific to folpet, the concomitant use of phthalic acid as a major biomarker of exposure to folpet should also be considered.

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

Table of Contents – Short abstract

To determine the kinetics of ring metabolites of captan and folpet in human matrices following an oral exposure, volunteers ingested 1 mg kg^{-1} of these two largely used fungicides. Blood samples and complete urine voids were collected at fixed time periods respectively over 72 h and 96 h following ingestion. The results show a relatively short half-life for the three studied metabolites as well as their sensitivity as biomarkers of exposure to captan and folpet.

INTRODUCTION

Captan and folpet are widely used in different crops to treat fungal diseases. Health effects were documented mostly from animal toxicity studies (US EPA, 1975, 1999). No direct toxicity was reported in humans, except allergic dermatitis, severe eye irritations and irritation of the nose and throat (Hayes, 1982; Lisi *et al.*, 1987; ACGIH, 1991; Edwards *et al.*, 1991; Trochimowicz *et al.*, 1991; Guo *et al.*, 1996; Tomlin, 1997; Costa, 2008; Gordon, 2010). Nonetheless, folpet was classified B2, or probable human carcinogen, by the U.S. Environmental Protection Agency (US EPA, 1975, 1999), based on an increased incidence of duodenum tumors in mice chronically exposed to high doses by gavage. Captan was also found to induce the same type of tumors in mice through the same mode of action and was thus initially categorized B2. However, in 2004, the US EPA revised the classification of captan and changed it to "not likely" (US EPA, 2004; Gordon, 2007), given that cancers were observed at doses several orders of magnitude higher than those encountered in occupational settings, which promoted proliferation of nascent tumors through cytotoxicity and cell hyperplasia.

The US EPA (1975, 1999) has also derived a reference dose (RfD) to prevent chronic effects of ingested captan and folpet in the general population. It was respectively established at 0.1 mg kg^{-1} of body weight (body wt) for captan (value estimated on the basis of a no-observed adverse effect level (NOAEL) of 12.5 mg kg^{-1} of body wt per day in a three-generation reproduction study in rats to which a safety factor of 100 was applied), and 0.16 mg kg^{-1} body wt per day for folpet (value assessed on the basis of a no-observed effect level (NOEL) of 10 mg kg^{-1} of body wt per day from a one-year toxicity study on dogs and a two-years toxicity and carcinogenicity study in rats to which a safety factor of 100 was applied) (Larsen, 1996).

With regard to captan and folpet metabolism (Fig. 1 and 2, respectively), it has been fairly well documented in *in vivo* studies in animals usually exposed to the radiolabelled compounds as well as *in vitro* studies, but human data are limited (Gordon, 2010). According to these studies, captan is broken down to 1,2,3,6-tetrahydrophthalimide (THPI) and thiophosgene (Wolfe *et al.*, 1976). Thiophosgene is an unstable metabolite with a very short half-life that reacts *in vivo* with thiol groups or several functional groups to form thiazolidine-2-thione-4-carboxylic acid (TTCA) (Wolfe *et al.*, 1976). THPI and TTCA have been proposed as biomarkers of captan exposure (DeBaun *et al.*, 1974; Krieger and Thongsinthusak, 1993). Both these metabolites can be quantified in human urine as evidenced by the only published urinary excretion time course study in two volunteers exposed to captan by the oral route (Krieger and Thongsinthusak, 1993). However, only THPI was repeatedly used as a biomarker in the few cross-sectional biomonitoring studies reported among workers exposed to captan (Winterlin *et al.*, 1984; Winterlin *et al.*, 1986; van Welie *et al.*, 1991; de Cock *et al.*, 1998; Krieger and Dinoff, 2000; Hines *et al.*, 2008). TTCA may also appear as a potential biomarker of captan exposure, but it is not specific to this fungicide and only van Welie *et al.* (1991) quantified it in the urine of exposed workers.

On the other hand, in *in vivo* experiments in animals and *in vitro* studies, folpet was shown to be first hydrolyzed to phthalimide (PI) and thiophosgene (Gordon *et al.*, 2001; Zainal and Que Hee, 2003; Canal-Raffin *et al.*, 2008; Gordon, 2010). Thiophosgene forms TTCA in the same way as captan, and PI is rapidly hydrolyzed in animals to unstable phthalamic acid, but also to phthalic

acid (Gordon, 2010). However, to our knowledge, there are no reported kinetic studies in humans for folpet to date.

Overall, there is a lack of detailed knowledge of the toxicokinetics of captan and folpet in humans necessary to interpret biomarker data in field studies and to relate biomarker data to health effects. Since interspecies differences in the kinetics of chemical substances are likely to occur (Krieger and Thongsinthusak, 1993; Poet and McDougal, 2002; Ngo *et al.*, 2010) and given the high doses administered in the animal studies on captan and folpet compared to occupational and environmental exposure levels, it is difficult to extrapolate *a priori* animal time course data to humans. As mentioned by Finley and Paustenbach (1997) and Woollen (1993), information obtained directly in humans will avoid some factors of uncertainty that are present in animal studies, and may give indications on the individual variability in metabolism and elimination of the studied substances. The aim to this study was thus to determine kinetics of captan and folpet metabolites in accessible biological matrices of orally exposed volunteers to help better assess biomonitoring data.

MATERIALS AND METHODS

Study design

A controlled kinetic time course study in accessible biological matrices of healthy subjects was conducted following an acute oral 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 ten male students aged from 20 to 30 years old, weighing 60 to 85 kg and measuring 169 to 184 cm in height. 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 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 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.

The volunteers ingested a dose of captan or folpet equivalent to 1 mg kg^{-1} body wt (n = 5 per group). 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 plastic cup and adjusted to the weight of each volunteer. The fungicide dose was mixed with 25 ml of orange juice and administered to the participants. The cup used for dosing was then rinsed with another 25 ml of orange juice and also administered to the participants.

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 on them 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 the 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.*, 2011a). Briefly, THPI and PI were isolated by adding 125 µl of THPI-d internal standard (1.59 µmol l^{-1}) in 3 ml of urine or 2 ml of plasma, conditioning the Oasis® solid phase extraction cartridge (Waters, Montreux, Switzerland) with 8 ml of

dichloromethane followed by 8 ml of methanol and 12 ml of water, and lastly by loading aliquots on SPE cartridges (in the case of plasma, following a denaturation of proteins). The analytes were then eluted from the column with 4 ml of dichloromethane. The solvent was evaporated to dryness under a gentle nitrogen flow at 40°C. The residues were resuspended in 500 µl of methanol 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 C_{18} 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 (v/v), and eluent B of 10% water and 90% acetonitrile (v/v). For THPI analysis, 10 µl of the sample were injected and elution was performed in 26 min at a flow rate of 0.8 ml min^{-1} using a solvent gradient starting at 90% eluent A for 3 min, followed by a linear gradient to 5% eluent A from 3 to 10 min, maintained at 5% eluent A from 10 to 13 min before running to initial conditions of 90% eluent A in 1 min for a 12-min re-equilibration of the column prior to subsequent injection. As for PI analysis, 10 µl of the sample were also injected and elution was performed in 33 min at a flow rate of 0.8 ml min^{-1} using an isocratic mode at 90% eluent A for 15 min and then ramping to 5% eluent A in 30 sec for a 4 min clean up at 5% eluent A prior to returning to initial conditions in 1 min to allow 12 min re-equilibration of the column. 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 peak 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.*, 2011b). 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 peak area. The analytical limit of detection was 60.2 nmol l^{-1} urine.

Calculations

The molar fraction of captan or folpet administered dose recovered in urine as THPI, PI or phthalic acid was calculated from the following equation:

$$\left[\frac{\left(\frac{Q_{urinary\ metabolite}}{MW_{metabolite}}\right)}{\left(\frac{Dose_{parent\ compound}}{MW_{parent\ compound}}\right)}\right] \times 100$$

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 (Hayes, 2008). The apparent elimination half-life ($t_{1/2}$) was then defined using the equation $t_{1/2} = 0.693\ k^{-1}$ (Hayes, 2008). All calculations were performed using MS Excel® 2007 software.

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:

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

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

$$MRT = \frac{AUMC}{AUC}$$

$$CL = \frac{Absorbed\ dose}{AUC}$$

$Vd = \frac{CL}{k}$ where k 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 ingestion of captan or folpet (1 mg kg^{-1} body wt) are presented in Fig. 3. 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 9.3 nmol l^{-1}. Following ingestion, plasma levels of THPI and PI increased progressively with peak levels of THPI being observed on average at time 10 h post-dosing as compared to 6 h post-dosing for PI. Subsequently, elimination phase of THPI and PI from plasma appeared monophasic (6-10 h to 72-h post-dosing) with a mean apparent elimination half-life of 15.7 and 31.5 h, respectively (Table 1). Table 2 presents the toxicokinetic parameters calculated from the THPI and PI time profiles in plasma and shows that THPI had a greater bioavailability and faster clearance rate than PI. As also shown in Table 2, PI metabolite in plasma represented only a small fraction of folpet dose. Nonetheless, mean residence time (MRT) of both THPI and PI was similar and in the order of one day, which is relatively short compared to other chemical compounds. THPI and PI also had similar relatively small apparent volume of distribution (V_d).

Time courses of THPI, PI and phthalic acid in urine

The time courses of THPI, PI and phthalic acid excretion rate in the urine of volunteers over a 96-h period following ingestion of 1 mg kg^{-1} body wt of captan or folpet are presented in Fig. 4. Peak levels were observed on average 9 h post-dosing for THPI, and between 3 and 12 h for PI and phthalic acid. Following peak excretion, elimination rate time courses evolved in parallel for PI and phthalic acid and was relatively slower than that of THPI, with mean apparent elimination half-lives calculated for the 24- to 96-h period post-dosing being 27.3, 27.6 and 11.7 h, respectively. Although the time courses of PI and phthalic acid was similar, phthalic acid was present in urine in 1 000-fold higher amounts than PI as shown in Fig. 4, and in Fig. 5 depicting the cumulative urinary excretion time courses of these metabolites. From the cumulative urinary excretion of PI and phthalic acid over the 96-h collection period post-dosing, it was calculated that 25% of the ingested dose of folpet was excreted in urine as phthalic acid as compared to only 0.02% as PI. Corresponding value for THPI was 3.5%.

Figure 6 shows that the time course of THPI in urine obtained in our study is similar to that obtained by Krieger and Thongsinthusak (1993) in volunteers orally exposed to the same dose of captan. In the current study, cumulative urinary excretion of THPI over the 96-h collection period post-treatment represented 3.5% of the administered dose, which is compatible with the value of 2.2% over the 72-h collection period in the study of Krieger and Thongsinthusak (1993). Similar to Krieger and Thongsinthusak (1993), elimination of THPI in urine was almost complete 96 h post-dosing.

Comparison of plasma and urinary time courses of THPI and PI

As expected for both THPI and PI, comparison of the plasma and urinary rate time courses in the studied volunteers (Fig. 3 and 4) shows that profiles evolved in parallel in the 10- to 72-h period

post-dosing. However, for both THPI and PI, plasma levels (nmol) were about 50-fold higher than urinary excretion rates (nmol h^{-1}), indicating that the transfer rate of THPI and PI from plasma to urine was approximately 0.02 per hour, corresponding to a half-life of ≈30 h.

DISCUSSION

This study allowed a better understanding of the kinetics of key biomarkers of exposure to captan and folpet. It provided novel human data on the kinetics of THPI in plasma, and PI and phthalic acid in plasma and urine, while confirming THPI urinary data of Krieger and Thongsinthusak (1993). Interestingly, several similarities were observed with available toxicokinetics of total radioactivity measured in rats orally exposed to labelled captan or folpet.

Results of the current study showed that the metabolite of captan, THPI, and the metabolites of folpet, PI and phthalic acid, had a rapid kinetics in humans after a single oral dose of 1 mg kg^{-1} body wt, since these metabolites were almost completely excreted over a 96-h period post-treatment. Peak levels in plasma were observed on average 10 h post-ingestion for THPI and 6 h post-dosing for PI while elimination half-life of THPI from plasma was in the order ≈15 h as compared to ≈30 h for PI.

Results of the current study also show negligible storage in tissues. Indeed, from the plasma time course, similar relatively small volume of distribution (V_d) were calculated for THPI and PI, suggesting these compounds remain mainly in the circulation and have limited distribution in body tissues, implying a low storage of these compounds in tissue. This is in line with animal studies showing negligible accumulation of THPI (Piccirillo, 2001) or phthalimide moiety (Couch *et al.*, 1977; Ackermann *et al.*, 1978) in tissues following oral or intraperitoneal administration of labelled captan or folpet.

Furthermore, although the time courses of PI and phthalic acid evolved in parallel, PI metabolite in plasma represented only a small fraction of folpet dose and only 0.03% of the folpet dose was recovered in urine as PI while 25% of the folpet dose was excreted in urine as phthalic acid over the 96-h period post-dosing. This is consistent with a rapid site-of-entry biotransformation of PI into phthalimic acid and phthalic acid once formed (Ackermann *et al.*, 1978; Canal-Raffin *et al.*, 2008), thus limiting the amounts of PI available for absorption in blood. It also shows that the acids formed in the GI following oral exposure are effectively absorbed.

Results obtained in the current study on the fraction of ingested dose recovered in urine as folpet metabolites is in accordance with mass-balance studies conducted in animals exposed to labelled folpet. When PI was orally administered to rats, about 80% of the administered dose was metabolized and excreted in urine as phthalamic acid while 7% was found as phthalic acid, and less than 1% of the dose was recovered as PI in urine (Chasseaud *et al.*, 1974). Phthalamic acid also represented the main metabolite (i.e. 80%) when labelled ^{14}C-folpet was orally administered to rats (Chasseaud, 1980). They however reported that this metabolite was unstable in urine.

In comparison with the folpet metabolites assessed in our study, on average 3.5% of the administered oral dose of captan was excreted as THPI in the urine of volunteers over the 96-h collection period. As shown in Fig. 6, these findings are similar to those obtained by Krieger and Thongsinthusak (1993) where 2-3% of the same orally administered dose of captan was recovered as THPI in the urine of a human volunteer over the 72-h period post-dosing. These human values are in the same range as those reported in a unique published animal study on the excretion of specific metabolites following non-labelled administration (van Welie *et al.*, 1991). Namely, van

Welie *et al.* (1991) reported that on average 0.7 to 5.4% of a single oral dose of 400, 815 or 1250 mg kg^{-1} of captan in Wistar rats were recovered in urine as THPI over the 0-24 h period post-dosing as compared to 2.8 to 9.1% over the 0-48 h period post-dosing, with lowest percentages observed at the highest dose. When non-labelled THPI itself was administered orally to rats, van Welie *et al.* (1991) also observed that on average only 4% of dose was recovered as THPI in urine over the 0-48 h period post-dosing.

It is to be noted that although a small percentage of orally administered captan in volunteers and rats was observed as THPI in urine, following oral administration of ring-labelled captan in animals, the majority of the radioactivity was recovered in urine (75 to 85% of administered dose) over a 24-h to 96-h period post-dosing, while only 7 to 12% of dose were excreted in feces over the same period of time. More precisely, Lappin and Havell (1990) reported that 81% of an oral dose of 10 mg kg^{-1} of ^{14}C-ring-captan were recovered in urine as labelled equivalents as compared to 8-9% in feces over a 96-h period post-dosing, and 97% of the dose were eliminated at this time. Similarly, Trivedi (1990) reported that, following an oral administration of 10 mg kg^{-1} of ^{14}C-ring-labelled captan, 75% of the administered dose were excreted in urine as ^{14}C-equivalents and 6.5% in feces over the 24-h period post-dosing.

Similarly to captan, following oral or intraperitonal administration of ring-labelled folpet, between 90 and 100% of the administered dose were excreted in the urine of rats over a 24-h period post-dosing. This is based on the studies of Wood *et al.* (1991) showing that 92% of an orally administered dose of 10 mg kg^{-1} of ^{14}C-labelled folpet in rats were recovered in urine as ^{14}C equivalents as compared to 6% in feces, and on the study of Couch *et al.* (1977) indicating that virtually 100% of an intraperitoneal dose of 6 mg kg^{-1} of ^{14}C-folpet were recovered in urine as ^{14}C equivalents and 1.7% in feces over the 24-h period post-dosing.

This confirms that, in humans as observed in animals (Lappin and Havell, 1990; van Welie *et al.*, 1991), studied PI and THPI metabolites are not the main metabolites of captan and folpet following oral exposure. As shown by our results and reported by van Welie *et al.* (1991), other metabolites of captan might be more representative than THPI, although the analytical method developed by our team for this metabolite was specific, accurate and sensitive. Following an oral administration of 10 or 500 mg kg^{-1} of ^{14}C-ring-labelled captan in Sprague-Dawley rats, Lappin and Havell (1990) identified six metabolites other than THPI in urine: 3-hydroxy-4,5-cyclohexene-1,2-dicarboximide (3-OH-THPI) (representing 42% of total urinary metabolites), 5-hydroxy-3,4-cyclohexene-1,2-dicarboximide (5-OH-THPI) (6%), 6-hydroxy-1-amido-2-carboxy-4,5-cyclohexene (3-OH-THPI-amic acid) (13%), 1-amido-2-carboxy-4,5-cyclohexene (THPAM) (7%), 4,5-dihydroxy-1,2-dicarboximide (4,5-diOH-THPI) (6%), and 4,5-epoxy-1,2-dicarboximide (THPI-epoxide) (5%), and two unidentified metabolites accounting for 4 and 2% of total urinary metabolites.

Overall, in a perspective of biomonitoring of exposure, THPI and PI appear as interesting biomarkers of recent exposure given that they are metabolites specific to captan and folpet, respectively, with relatively short half-lives. However, they represent only a small percentage of the orally administered dose in our study, such that their sensitivity as biomarkers of exposure in workers and even more in the general population remains to be more extensively verified. Other metabolites of captan and folpet appear quantitatively more important in human urine than THPI or PI. The most important urinary metabolite of captan in rats was found to be 3-OH-THPI (Lappin and Havell, 1990); it would be interesting to quantify this metabolite in human urine since the

current study indicates that the metabolism and excretion of captan metabolites may be similar in rats and humans. Furthermore, this human study in line with the rat studies of Chasseaud *et al.* (1974, 1980) showed that phthalamic or phthalic acid appear as quantitatively more important biomarkers than PI while urinary excretion time courses are similar for phthalic acid and PI. Nonetheless, phthalic acid is not a metabolite specific to folpet; it is also a derivative of phthalates, which are ubiquitous molecules in our environment (Blount *et al.*, 2000; Silva *et al.*, 2007). Given these considerations, perhaps the best biomonitoring strategy, to assess occupational or environmental exposure, would be to measure multiple metabolites of captan or folpet and to perform repeated measurements in time.

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Table 1. First-order apparent elimination half-lives of THPI and PI in human plasma and of THPI, PI and phthalic acid in human urine following ingestion of 1 mg kg^{-1} of captan or folpet

Metabolites	Matrices	Mean first-order elimination half-life (h)[a] (n=5)	Coefficient of determination (R^2)
THPI	Plasma	15.7	0.99
	Urine	11.7	0.98
PI	Plasma	31.5	0.84
	Urine	27.3	0.86
Phthalic acid	Urine	27.6	0.82

[a] The 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 (10- to 72-h period) or urine excretion rate time course (24- to 96-h period) for each metabolite

Table 2. Toxicokinetic parameters for THPI and PI in plasma of volunteers following ingestion of 1 mg kg^{-1} of captan or folpet

Model parameters	First order toxicokinetic values			
	THPI		PI	
	Mean	SD	Mean	SD
	(n=5)		(n=5)	
AUC [(nmol x h l^{-1}) kg^{-1}]	1 652	289	13.4	3.3
AUMC [(nmol x h^2 l^{-1}) kg^{-1}]	40 647	13 533	397	117
MRT (h)	23.9	4.0	29.4	2.2
CL (l h^{-1})	0.18	0.03	0.09	0.02
V_d (l)	3.4	0.6	4.3	1.1

Captions to figures

Figure 1. Captan metabolism according to *in vivo* studies in animals administered ^{14}C- or ^{15}S-radiolabelled doses (Lappin and Havell, 1990; Krieger and Thongsinthusak, 1993)

Figure 2. Folpet metabolism according to *in vivo* studies in animals and *in* vitro studies (Ackermann *et al.*, 1978; Wood *et al.*, 1991; US EPA, 1999; Canal-Raffin *et al.*, 2008; Gordon, 2010)

Figure 3. Time courses of THPI and PI in plasma of volunteers (expressed as nmol) over a 72-h period following ingestion of 1 mg kg^{-1} of captan or folpet. Each point represents mean and vertical bars are standard deviations (n = 5).

Figure 4. Time courses of THPI, PI and phthalic acid excretion rate in urine (expressed as nmol/h) of volunteers over 96-h period following ingestion of 1 mg kg^{-1} of captan or folpet. Each point represents mean and vertical bars are standard deviations (n = 5).

Figure 5. Time courses of THPI, PI and phthalic acid cumulative excretion in urine (expressed as nmol) of volunteers over a 96-h period following ingestion of 1 mg kg^{-1} of captan or folpet. Each point represents mean and vertical bars are standard deviations (n=5).

Figure 6. Comparison of the time course of THPI urinary excretion rate (expressed as a % dose/h) in volunteers of the present study with that obtained by Krieger and Thongsinthusak (1993) following ingestion of 1 mg kg^{-1} of captan. Each point represents mean and vertical bars are standard deviations (n = 5).

Figure 1.

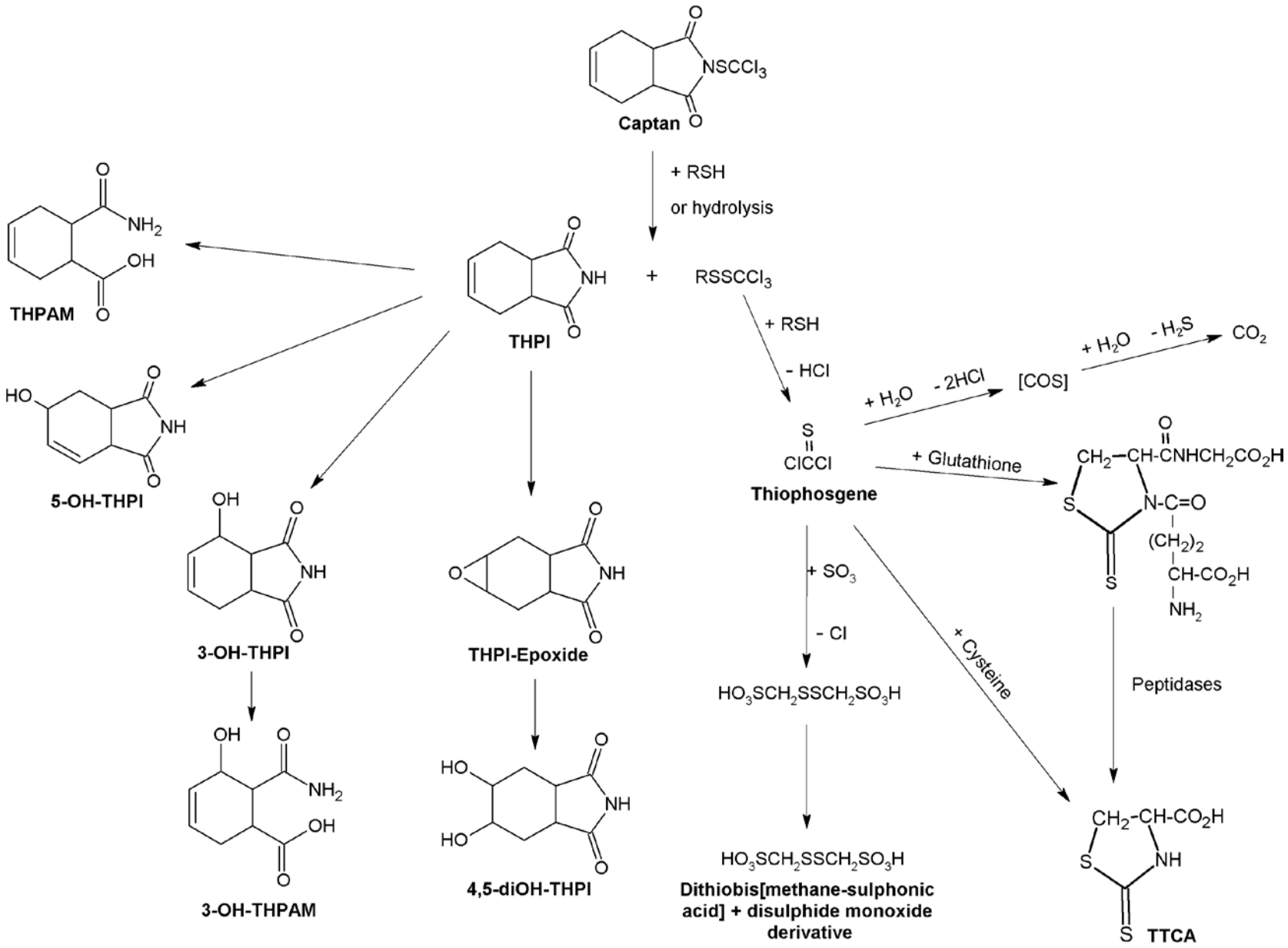

Captan
+ RSH
or hydrolysis
THPAM
THPI
+ RSSCCl3
+ RSH
- HCl
5-OH-THPI
3-OH-THPI
THPI-Epoxide
Thiophosgene
+ H2O - 2HCl
[COS]
+ H2O - H2S
CO2
+ Glutathione
+ SO3
- Cl
HO3SCH2SSCH2SO3H
+ Cysteine
Peptidases
3-OH-THPAM
4,5-diOH-THPI
HO3SCH2SSCH2SO3H
Dithiobis[methane-sulphonic acid] + disulphide monoxide derivative
TTCA

Figure 2.

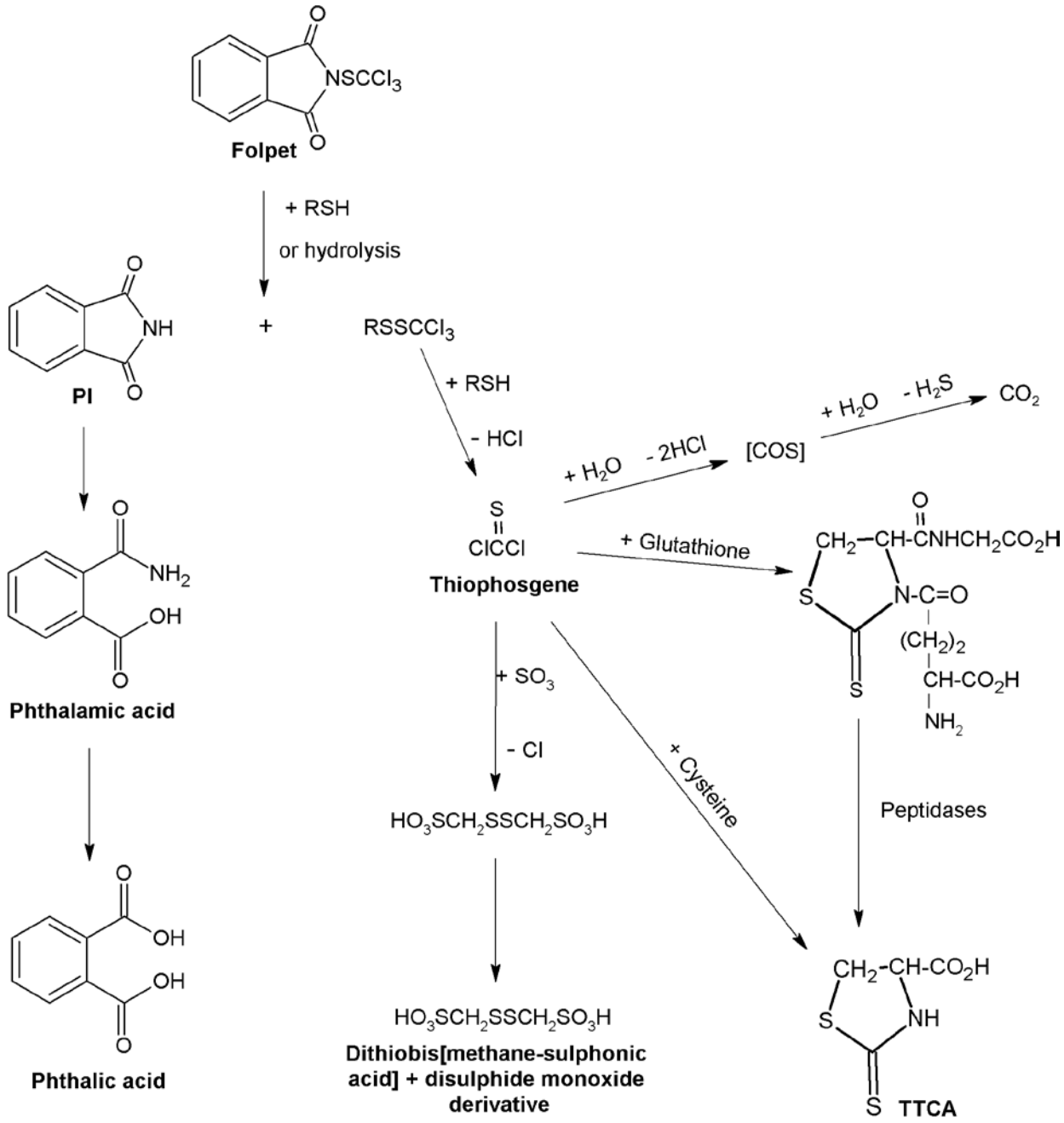

Folpet
+ RSH
or hydrolysis
PI
+
RSSCCl3
+ RSH
- HCl
+ H2O
- 2HCl
[COS]
+ H2O
- H2S
CO2
Thiophosgene
+ Glutathione
Phthalamic acid
+ SO3
- Cl
HO3SCH2SSCH2SO3H
+ Cysteine
Peptidases
Phthalic acid
HO3SCH2SSCH2SO3H
Dithiobis[methane-sulphonic acid] + disulphide monoxide derivative
TTCA

Figure 3.

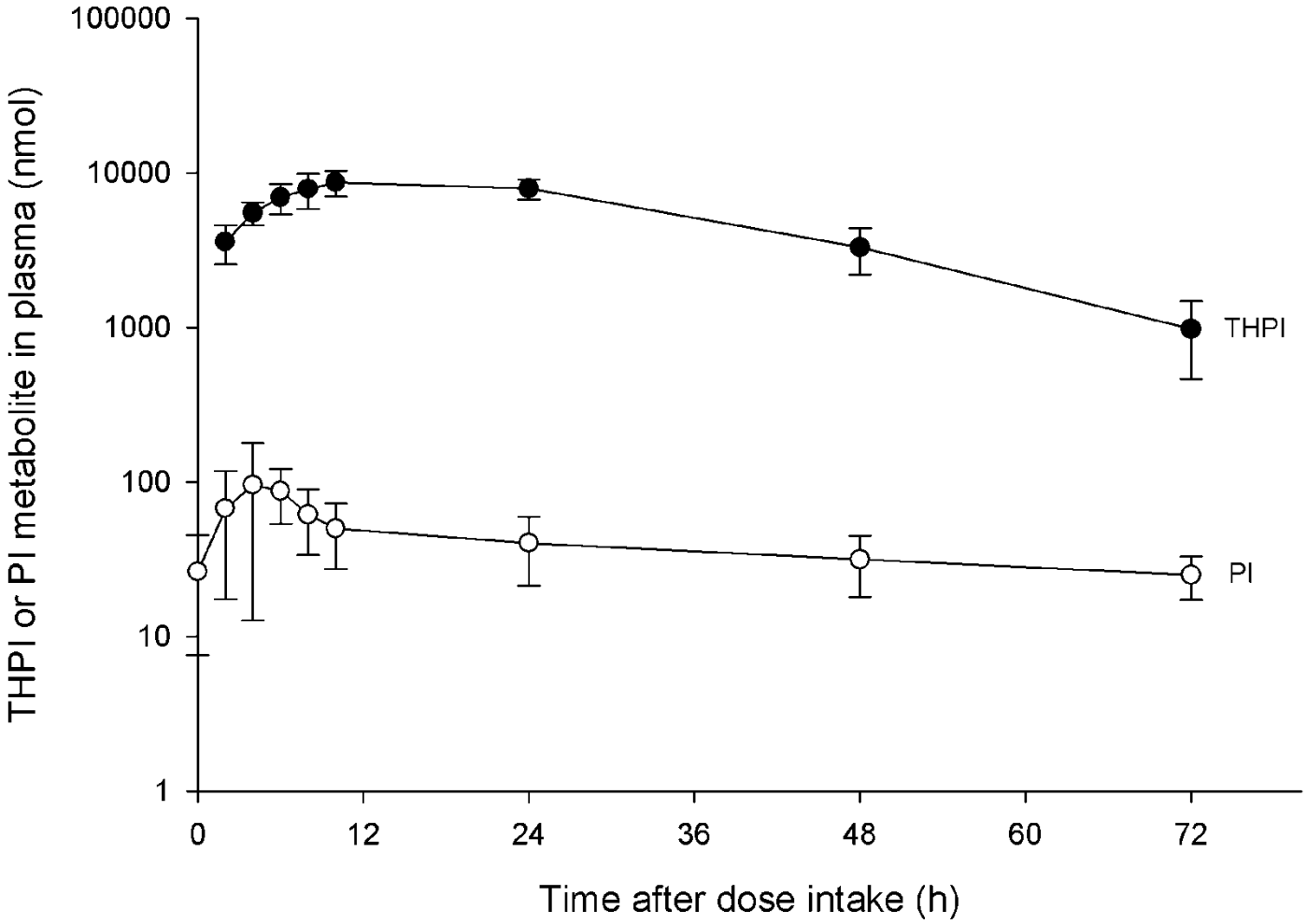

100000
10000
1000
100
10
1
THPI or PI metabolite in plasma (nmol)
0
12
24
36
48
60
72
Time after dose intake (h)
THPI
PI

Figure 4.

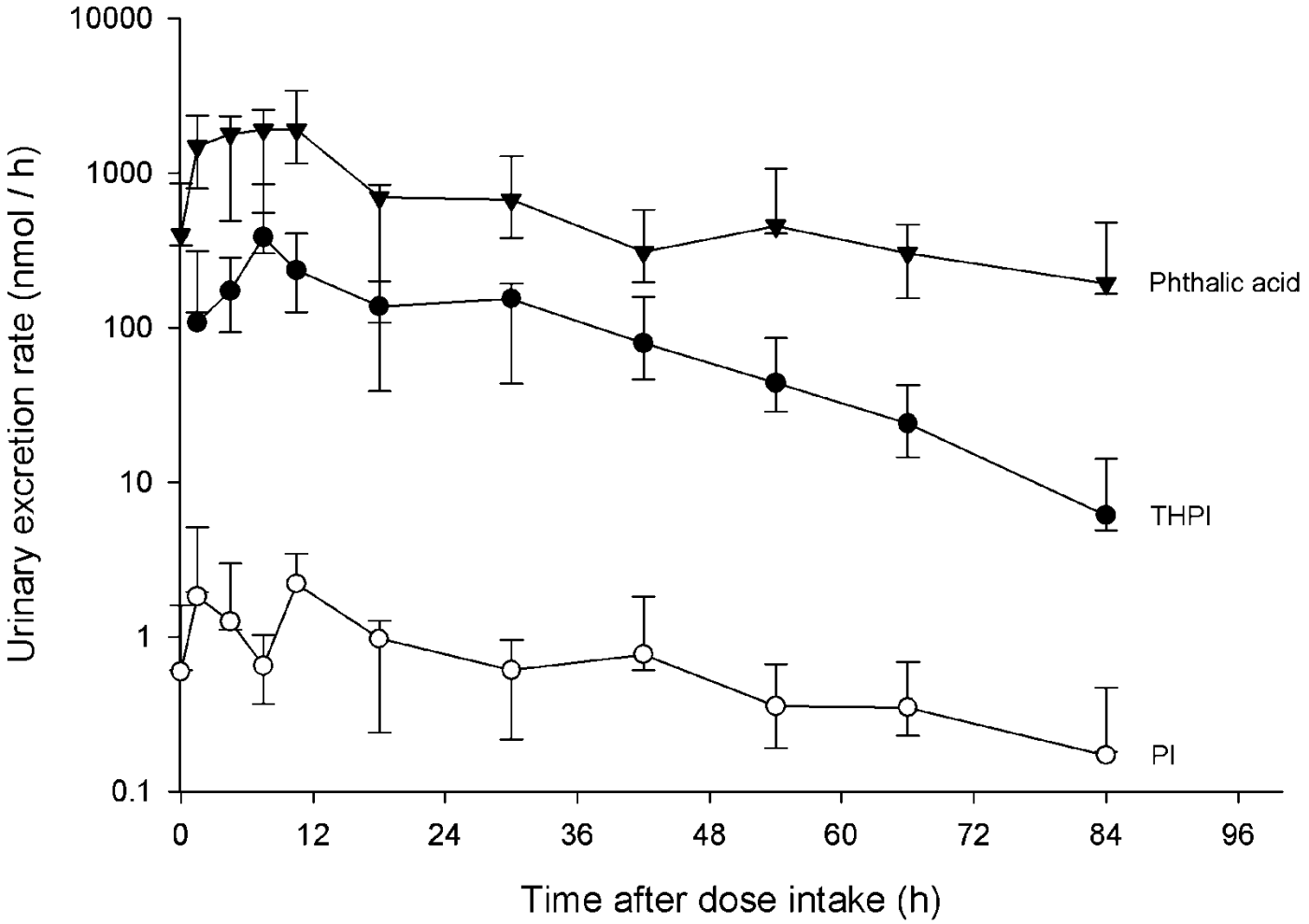

10000
1000
100
10
1
0.1
Urinary excretion rate (nmol / h)
0
12
24
36
48
60
72
84
96
Time after dose intake (h)
Phthalic acid
THPI
PI

Figure 5.

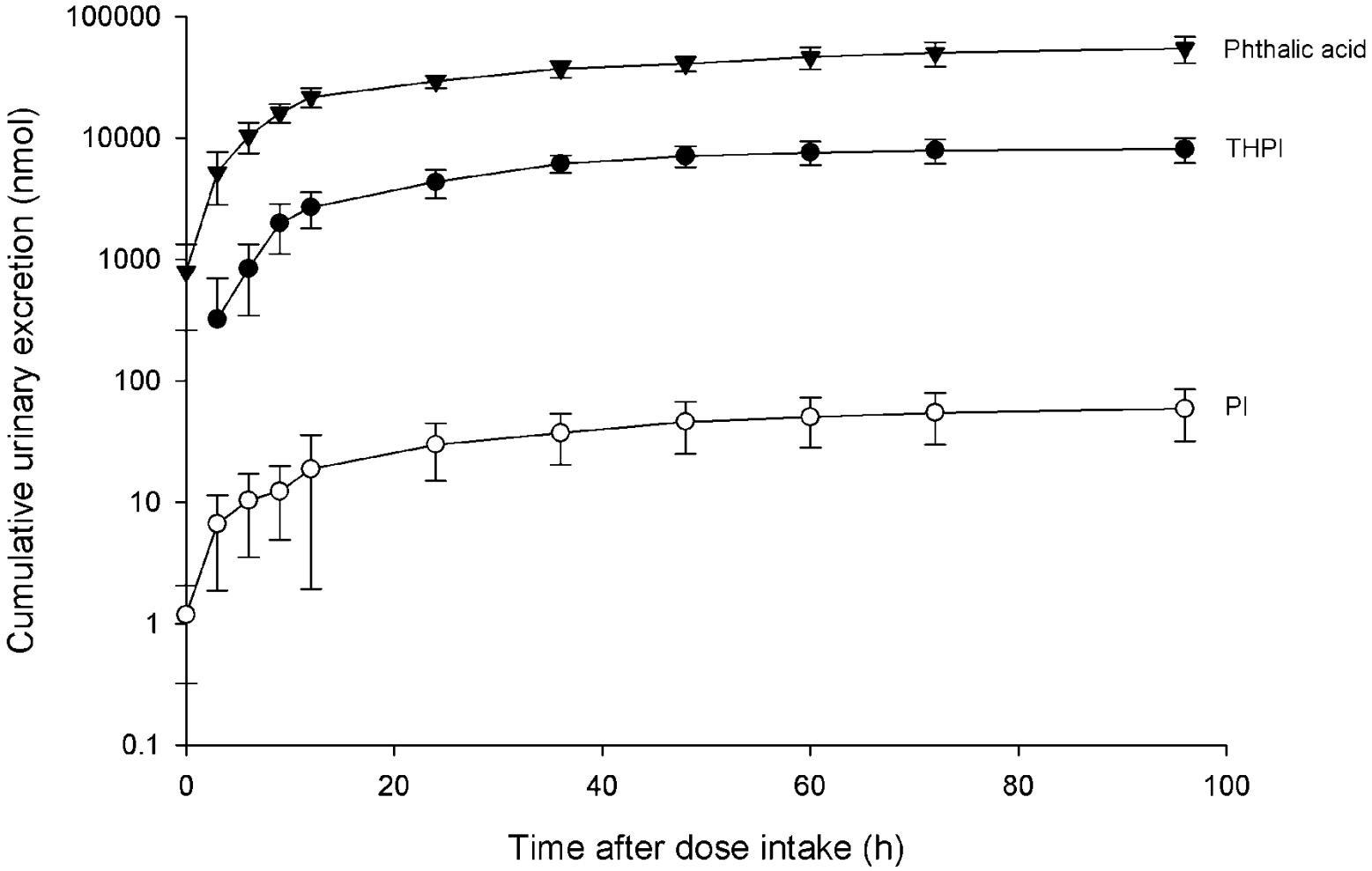
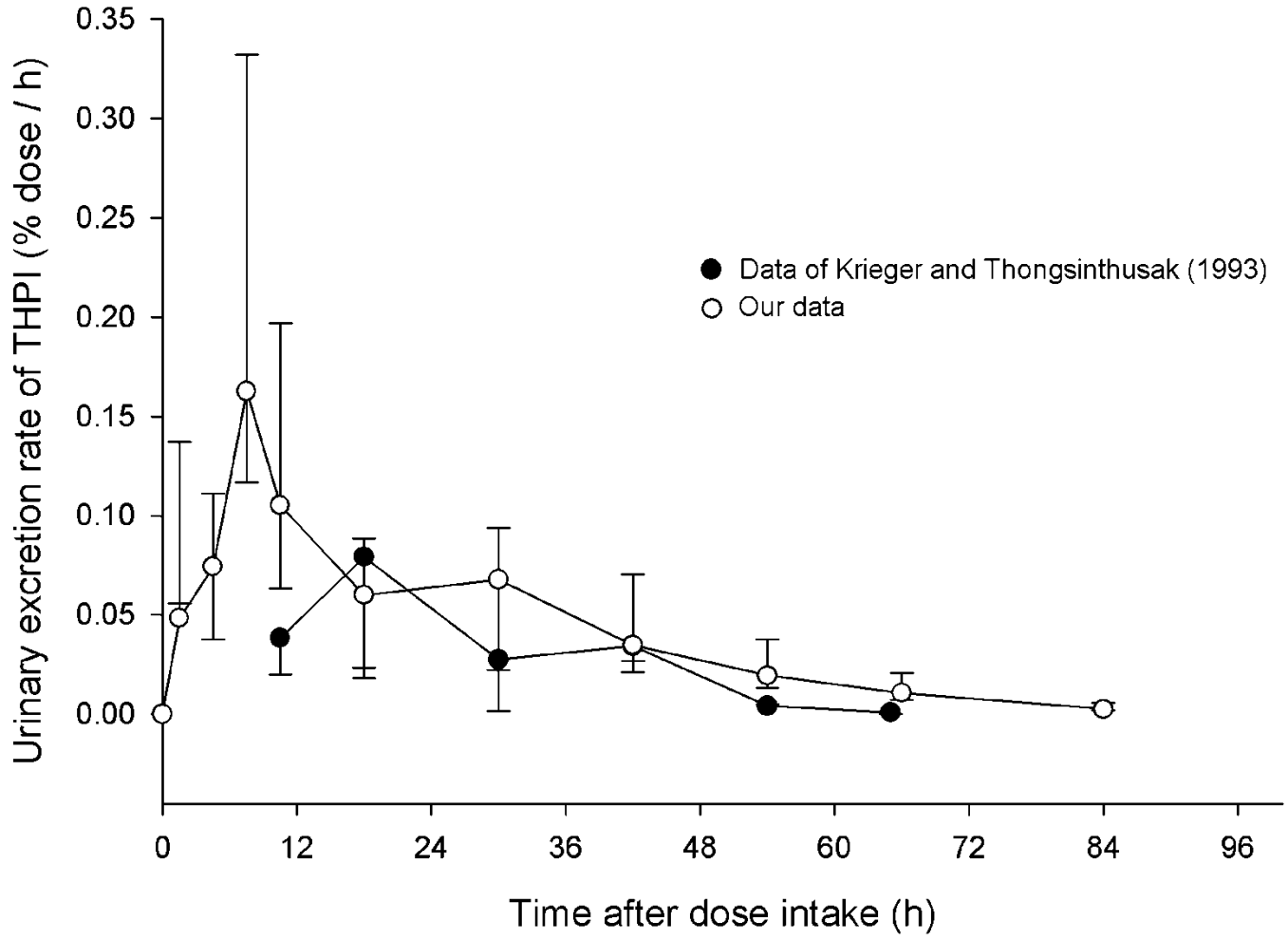

100000
10000
1000
100
10
1
0.1
Cumulative urinary excretion (nmol)
0
20
40
60
80
100
Time after dose intake (h)
Phthalic acid
THPI
PI

Figure 6.


Urinary excretion rate of THPI (% dose / h)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
12
24
36
48
60
72
84
96
Time after dose intake (h)
Data of Krieger and Thongsinthusak (1993)
Our data

Supplemental table

Average levels of THPI, PI and phthalic acid quantified in plasma and in urine at different points time in volunteers orally exposed to 1 mg kg^{-1} of captan or folpet.

Time (h)[b]	Concentrations (nmol ml^{-1})[a]				
	THPI[c] (mean ± SD)		PI[d] (mean ± SD)		Phthalic acid[e] (mean ± SD)
	Plasma	Urine	Plasma	Urine	Urine
0	0.004 ± 0.01	0	0.009 ± 0.004	0.005 ± 0.003	2.96 ± 1.65
2	1.14 ± 0.29		0.021 ± 0.012		
4	1.76 ± 0.19		0.029 ± 0.020		
3		0.69 ± 0.27		0.020 ± 0.018	16.06 ± 5.09
6	2.21 ± 0.38	1.41 ± 0.24	0.027 ± 0.007	0.011 ± 0.007	17.27 ± 3.40
8	2.51 ± 0.52		0.019 ± 0.006		
9		1.70 ± 0.37		0.007 ± 0.005	21.42 ± 11.0
10	2.77 ± 0.34		0.016 ± 0.005		
12		2.06 ± 0.59		0.023 ± 0.033	15.82 ± 6.42
24	2.62 ± 0.47	1.89 ± 0.41	0.013 ± 0.005	0.019 ± 0.008	13.17 ± 3.79
36		1.36 ± 0.31		0.011 ± 0.004	13.69 ±6.37
48	1.07 ± 0.39	0.94 ± 0.30	0.010 ± 0.003	0.018 ± 0.013	6.51 ± 3.15
60		0.54 ± 0.24		0.007 ± 0.002	8.67 ± 8.81
72	0.31 ± 0.17	0.34 ± 0.18	0.008 ± 0.003	0.007 ± 0.003	6.18 ± 2.41
96		0.13 ± 0.07		0.005 ± 0.003	5.74 ± 4.62

a Urinary concentrations observed following sample processing as described in Materials and Methods.

b Fixed time periods (expressed in hour) of urine and blood collections following fungicide ingestion.

c Metabolite of captan.

d Metabolite of folpet.

e Metabolite of folpet.