Ethylene oxide in ethylene-exposed mice, rats, and humans

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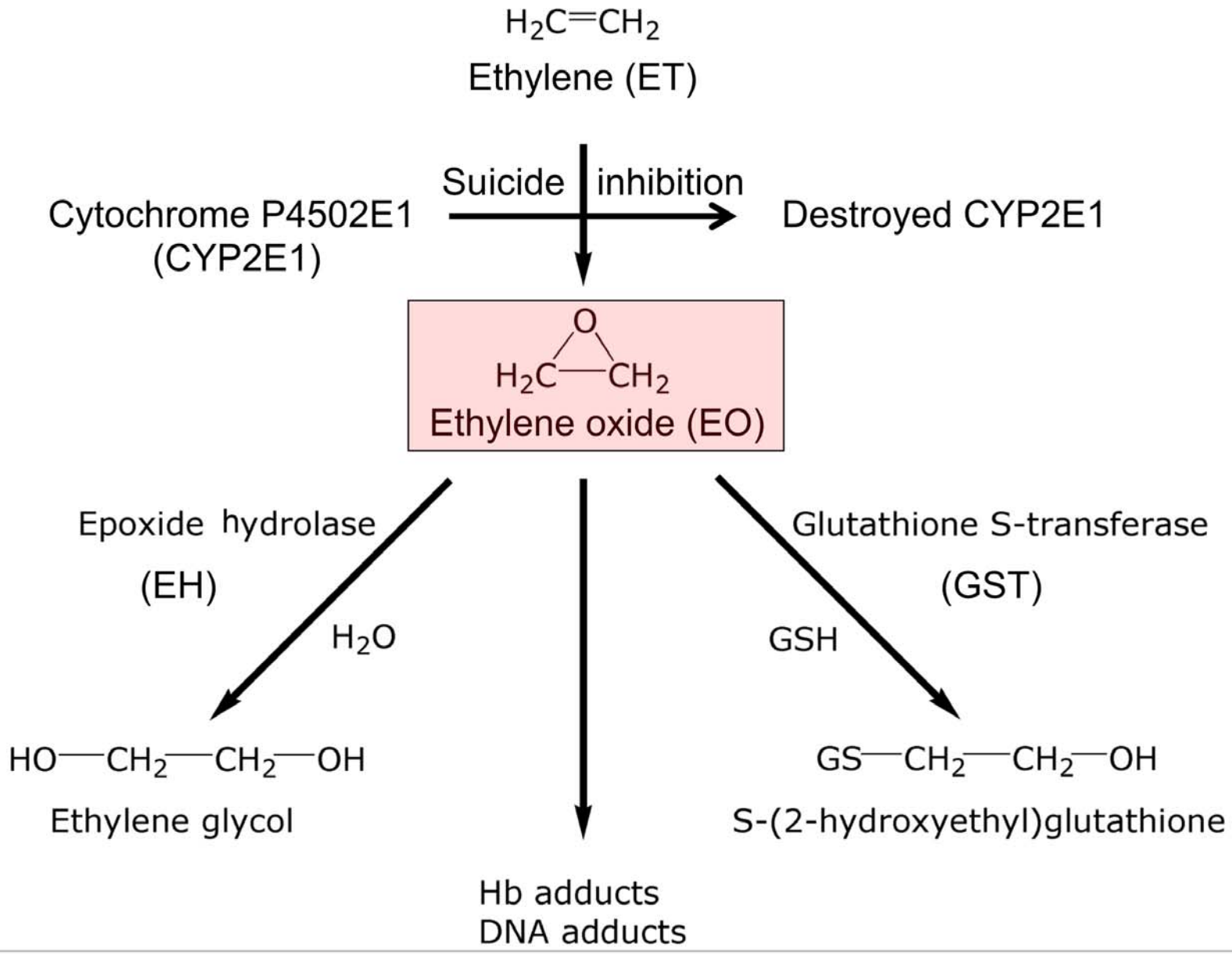
Metabolic fate of ethylene



Earlier work on the EO burden in ET-exposed rodents and humans

There is one physiological toxicokinetic (PT) model for inhaled ET, inhaled EO, and EO formed metabolically from ET (Csanády et al., 2000).

A series of data had been identified that would have been required for improving the PT model and its validation:

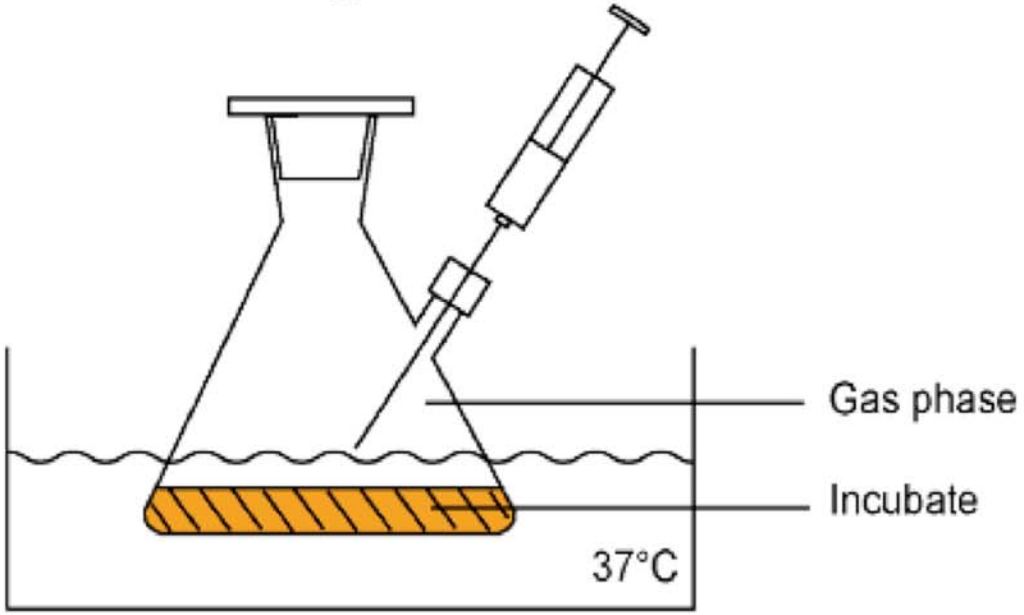
- **data on the mechanism of the EO formation**
- **data on EO blood levels in ET-exposed mice, rats, and humans**

The present work was done to fill these gaps.

Part 1

Determination of the kinetic parameters of the ET- and EO-metabolizing enzymes in liver cell fractions of mice, rats, and humans

Experimental procedure for obtaining the kinetic parameters of ET and EO using subcellular liver fractions



Microsomal or cytosolic suspensions with or without specific cofactors were incubated in closed all-glass vessels in the presence of defined amounts of gaseous ET or EO. Incubations were done in a shaking water bath for up to 65 min.

Concentration-time courses of EO in air were monitored by gas chromatography.

Microsomes were pooled from the livers of 50 male B6C3F1 mice, 10 male F344 rats, or 25 humans. Cytosol was pooled from livers of 50 mice, 10 rats, or 11 humans. Cytosol was also prepared individually from livers of 13 humans.

Results

Kinetics of CYP2E1-mediated elimination of ET (determined from the formation of EO):

V_{max} (nmol/min/mg protein): mouse (0.57) > rat (0.40) > human (0.22)

K_m (mmol/L suspension): rat (0.03) > human (0.01) > mouse (0.009)

Rate constants (min^{-1}) for suicide inhibition of CYP2E1 (reflecting the ET-mediated N-alkylation of the prosthetic heme group):

human (0.1) > rat (0.07) > mouse (0.06)

Results continued

Microsomal EH activity toward EO:

very low in rodents, near to nonenzymatic hydrolysis;

high in humans (V_{max} = 14.4 nmol/min/mg protein, K_m = 12.7 mmol/L suspension)

Cytosolic GST activity toward EO:

V_{max}/K_m (µl/min/mg protein): mouse (28) >> rat (5.3) > human (0.0 - 2.9)

Conclusion to Part 1

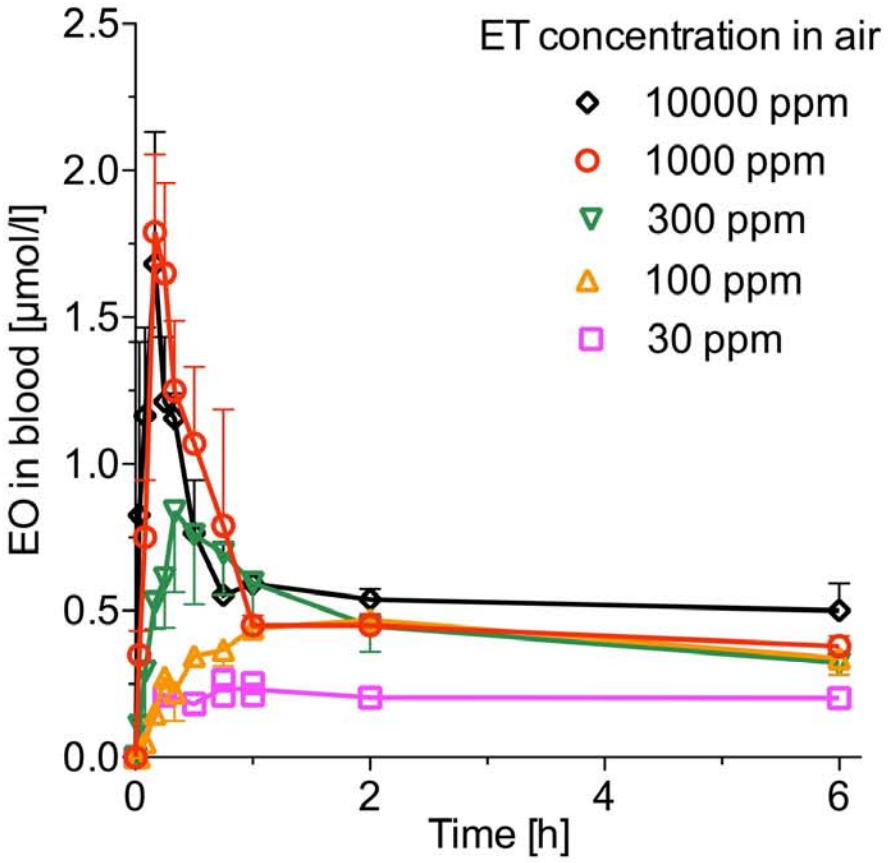
The toxicokinetic parameters required for improving the PT model for ET and EO in the three species are now available.

The studies presented in Part 1 have been published in Li et al. (2011).

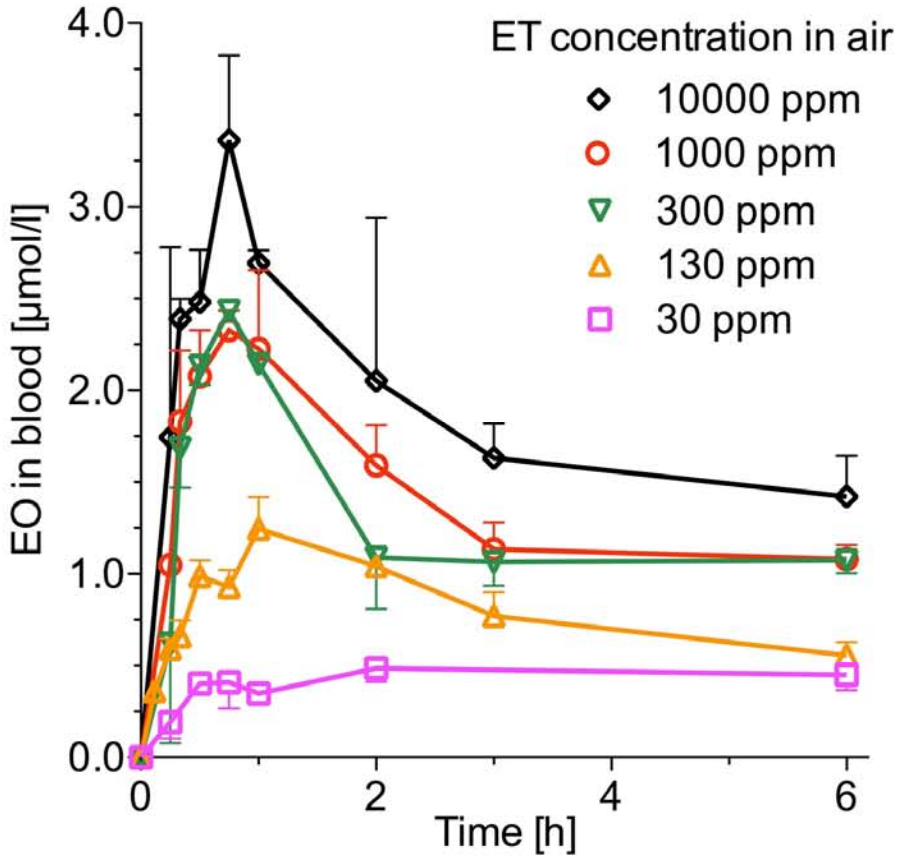
Part 2

EO in blood of ET-exposed mice, rats, and humans

EO in venous blood of mice and rats exposed to constant concentrations of ET in air



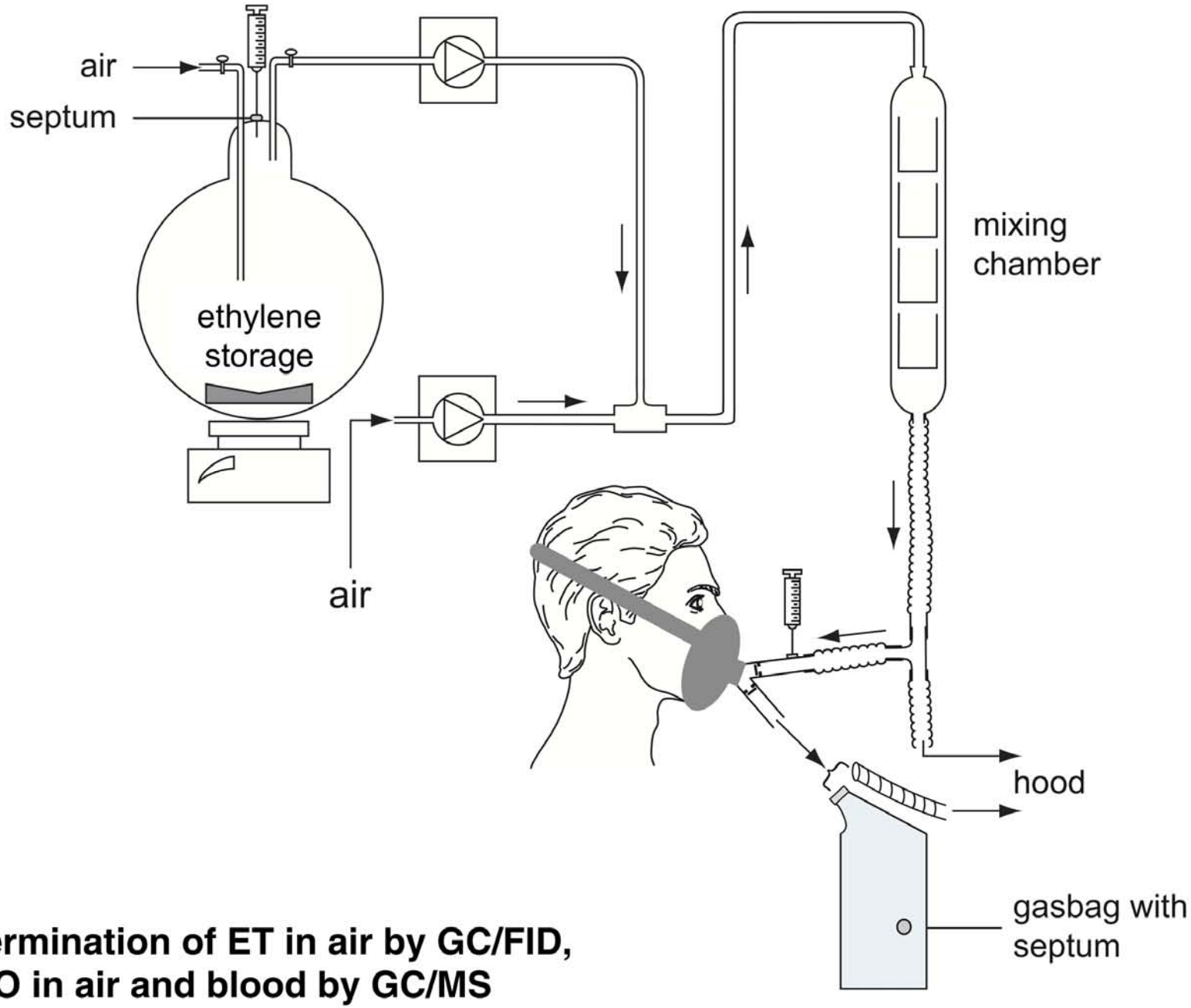
Male B6C3F1 mice



Male F344 rats

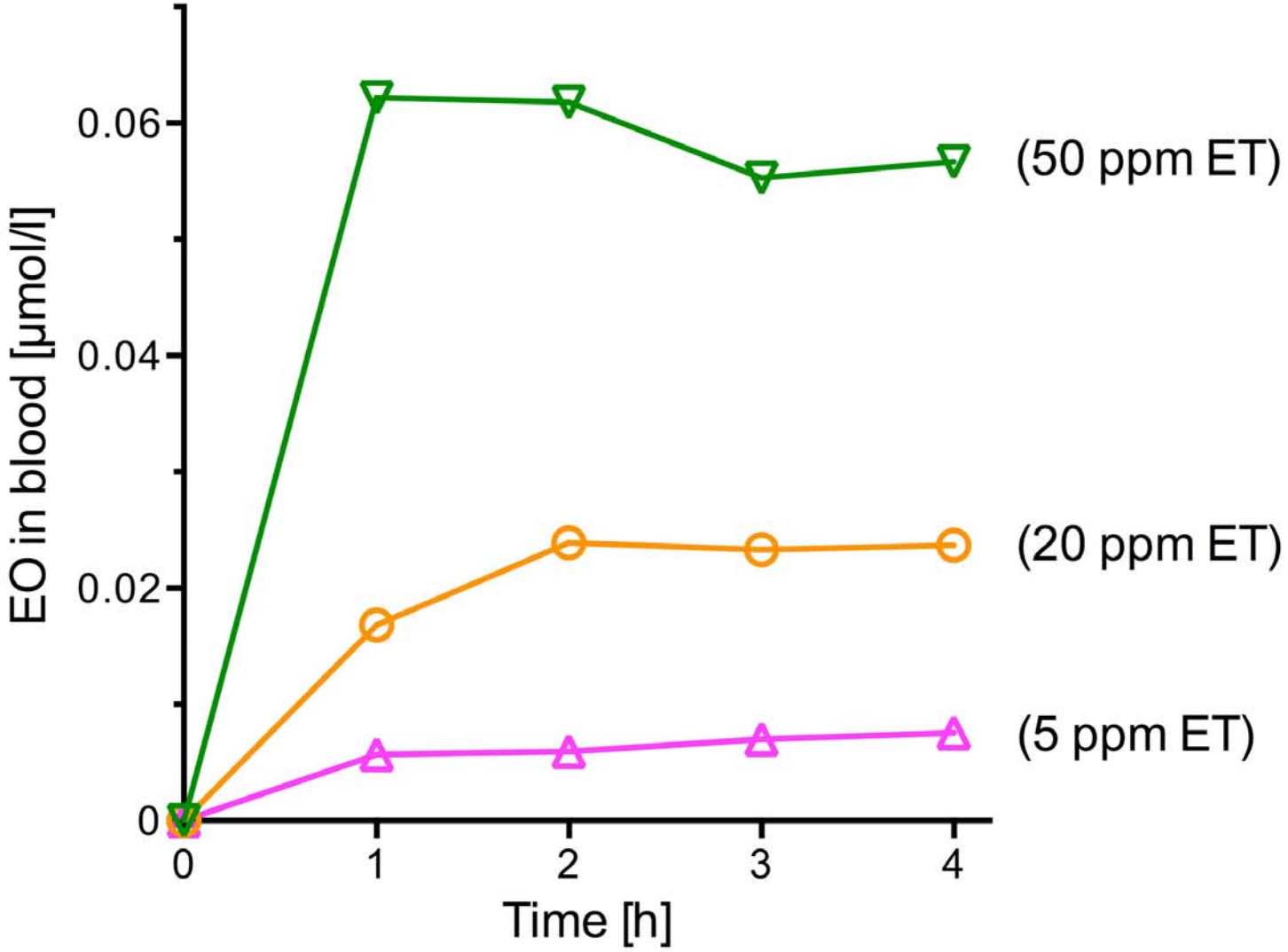
Determination of ET in air by GC/FID, of EO in blood by GC/MS

Human exposure system

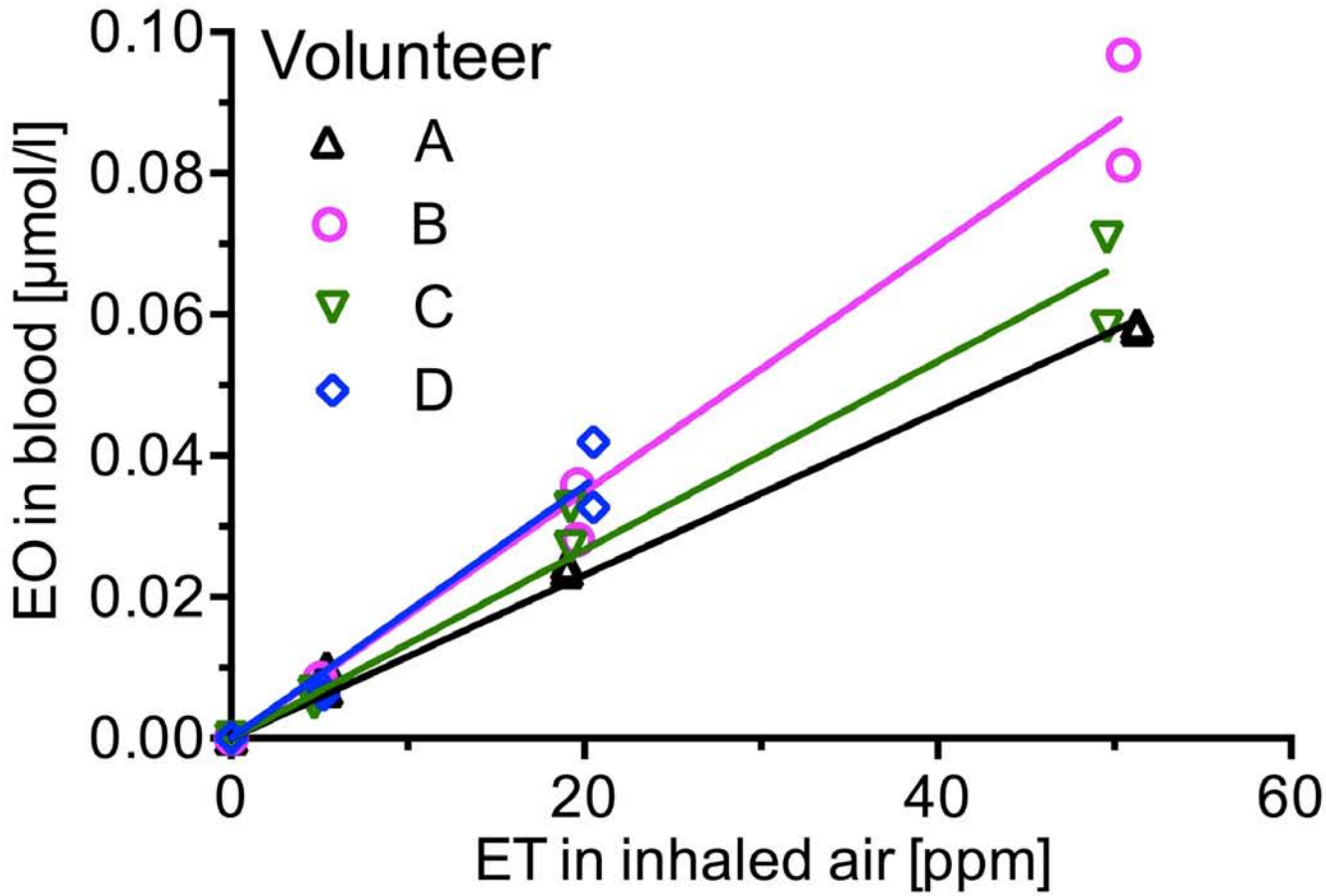


Determination of ET in air by GC/FID,
of EO in air and blood by GC/MS

EO in venous blood of a volunteer exposed to constant concentrations of ET in air



Plateau levels of EO in venous blood of four volunteers exposed for 4 h to constant concentrations of ET in air



Concentrations of ET were about 5, 20, and 50 ppm. One of the doubled EO data points at each chosen ET concentration was measured in blood, the other one calculated from the concentration of EO in exhaled air. Lines were obtained by linear regression through the origin. Volunteer D was not exposed to 50 ppm of ET.

Interspecies differences in blood levels of EO resulting from exposure to ET

At equal ET concentrations of up to 20 ppm, blood levels of EO at plateau are about 9-fold higher in rats and 4-fold higher in mice than in the human (volunteer D) with the highest EO burden.

The studies presented in Part 2 have been published in Filser et al. (2013).

Part 3

A physiological toxicokinetic (PT) model for ET and its metabolite EO in the rat

PT model for inhaled ET, inhaled EO, and metabolically produced EO

The structure of the PT model is based on the model of Csanády et al. (2000).

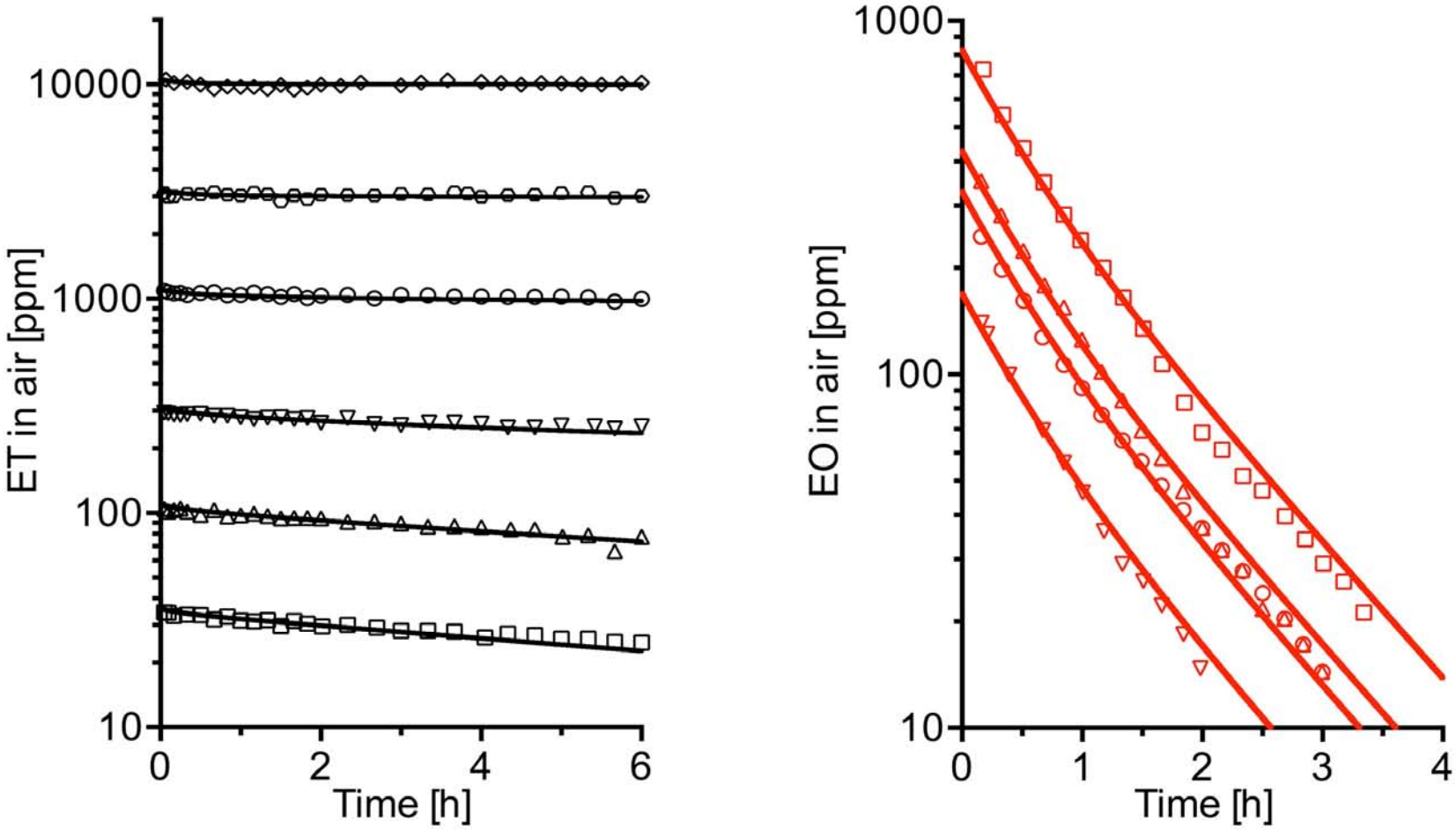
Major extensions

Incorporated in the PT model are now

- **turnover of CYP2E1,**
- **suicide inactivation of CYP2E1 by ET.**

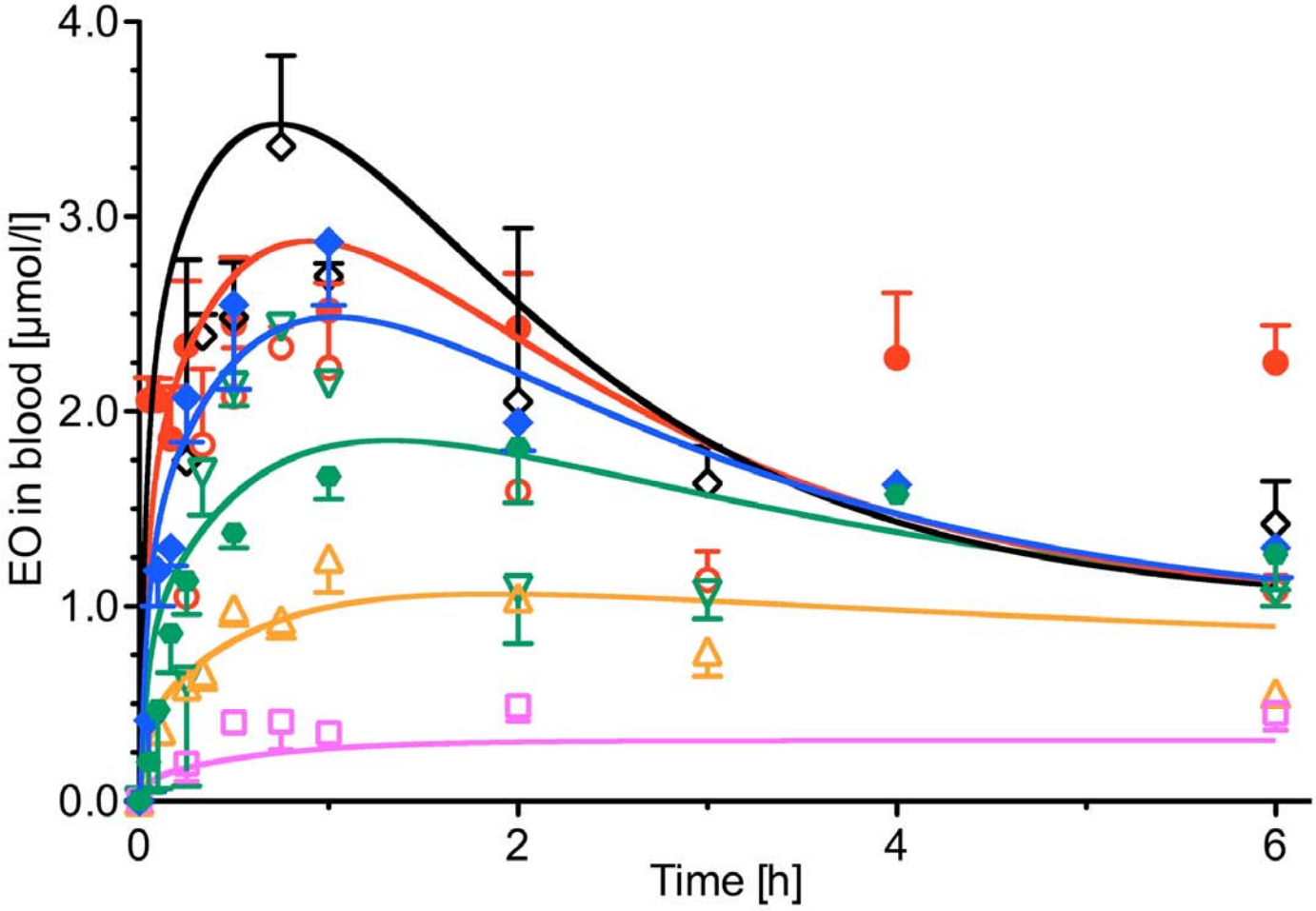
Most biochemical parameters have been derived from in-vitro data.

ET (left) or EO (right) in the air of closed chambers containing ET-exposed (left) or EO-exposed (right) male F344 rats



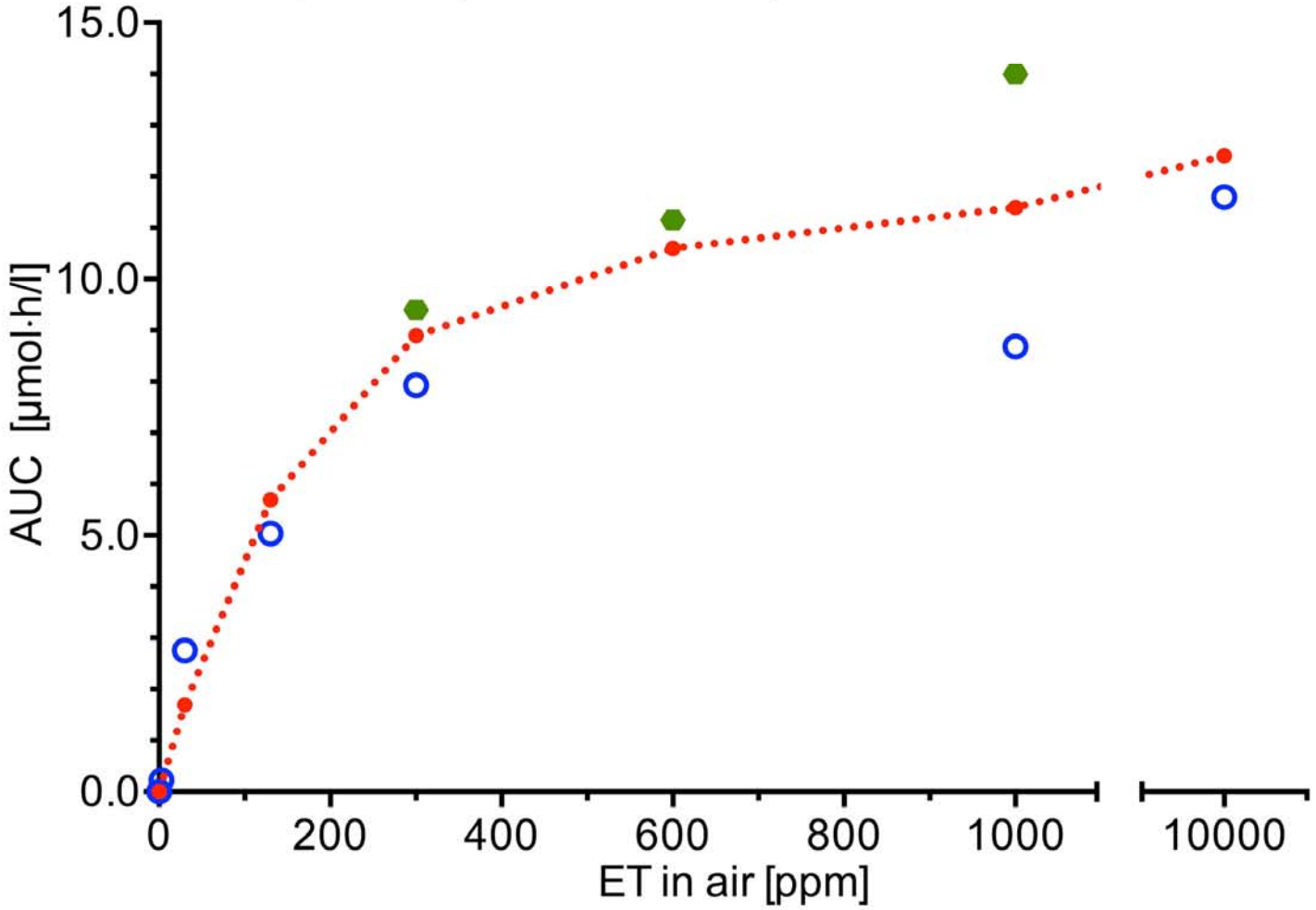
Lines, PT-model predictions; symbols, measured (ET, own data; EO, data of Krishnan et al., 1992)

EO in venous blood of ET-exposed male F344 rats; measured (symbols) and predicted by the PT model (lines)



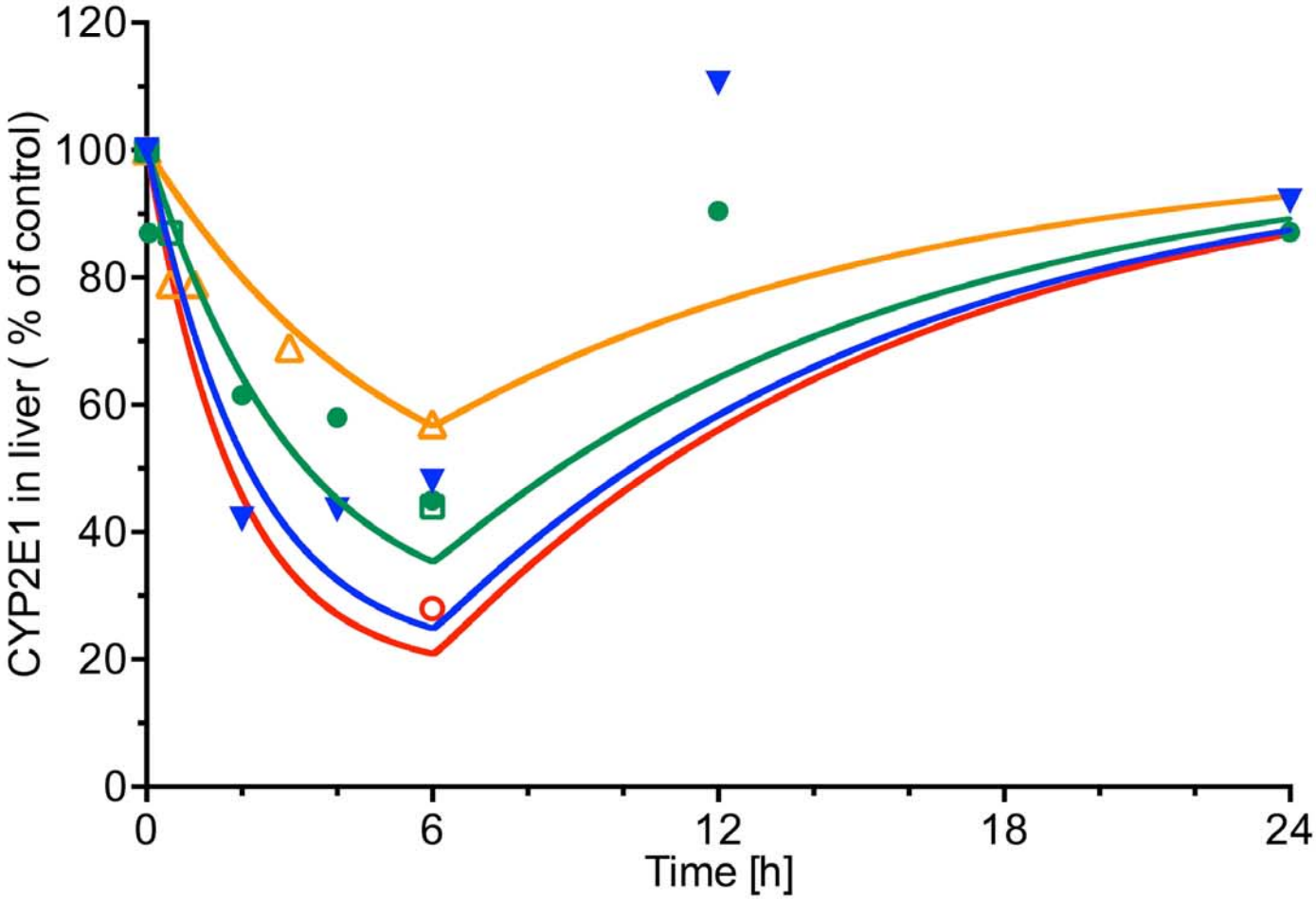
ET exposure concentrations, 10000, 1000, 600, 300, 130, and 30 ppm; open symbols, Filser et al. (2013); filled symbols, Fennell et al. (2004)

Area under the concentration-time curve of EO in venous blood of rats* (AUC+) versus exposure concentration of ET



*: male F344 rats; +: exposure time: 6 h. Dashed line and red symbols, predicted by the PT model; blue symbols, measured (Filser et al., 2013); green symbols, measured (Fennell et al., 2004)

PT-model predicted content (lines) and measured activity (symbols) of CYP2E1 in livers of ET-exposed male F344 rats



Single 6-h exposures to ET concentrations of 130, 300, 600, and 1000 ppm; open symbols, own data using chlorzoxazone; filled symbols, Fennell et al. (2004) using 4-nitrophenol

Summary of Part 3 and Outlook

The PT model has been validated for the ET- or EO-exposed rat.

The model predicts concentration-time courses of inhaled ET, inhaled EO and EO in venous blood of ET-exposed rats in agreement with measured data.

It also predicts the suicide inhibition of CYP2E1 and its turnover in rats in agreement with measured data.

After extending the model for mice and humans, it will be a useful tool for assessing risks of ET or EO.

Colleagues and collaborators involved in this work

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