Rijksinstituut voor Volksgezondheid en Milieu

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

RIVM-RIKILT FRONT OFFICE FOOD SAFETY

MODELLING OF THE TRANSFER OF MELAMINE FROM FEED TO PIG TISSUES

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Subject
Transfer modelling of melamine in feed to organs and plasma of pigs.

Research questions
1) Can the PBPK model as described by Buur *et al.* (2008) successfully be applied to estimate concentrations of melamine in meat, liver, kidney and plasma? What are the modifications or assumptions to be made?

2a) Can you by using the PBPK model estimate the concentration of melamine in muscle meat, liver and kidney of pigs (40 kg) fed twice daily 1 kg of a ration containing 500 mg melamine per kg feed (12.5 mg/kg body weight)? If so, can you estimate the corresponding transfer factors?

Conclusion
1) The PBPK model as published by Buur *et al*. (2008) can well be used to simulate the transfer of melamine to plasma, liver and muscle of pigs exposed to this compound via feed. However, it appeared that calculation including renal clearance based on free plasma melamine concentration rather than total plasma concentration was needed to describe the available experimental porcine data. The fact that this modified PBPK model was obtained from interspecies scaling from the rat to pigs may suggest that it could be applicable beyond these two species.

2) When this modified model was applied to a case in which pigs are repeatedly exposed to 12.5 mg melamine/kg body weight from feed, muscle, liver, kidney and plasma concentrations (mg/kg) ranging from 3.5 to 8.7 (muscle), 7.2 to 18.9 (liver), 10.8 to 26.8 (kidney) and 4.9 to 12.3 (plasma) could be calculated. Corresponding transfer factors (defined as tissue concentration/feed

concentration*100%) were 0.7 to 1.7% (muscle), 1.6 to 3.8% (liver), 2.2 to 5.4% (kidney) and 1.0 to 2.5% (plasma).

Introduction

Melamine may be transferred from feed to animals, thereby leading to a potential health risk for humans following the consumption of contaminated pig muscle, liver or kidney tissue. The magnitude of the exposure depends on the actual feed-to-food transfer of melamine in pigs. Because experimental data are scarce kinetic transfer modelling may be useful to calculate such transfer. This report applies such modelling in the case of melamine contamination of pig feed.

Basic melamine kinetics are as follows (Mast *et al.*; 1983);. In the rat 90% of the administered melamine is excreted with the urine within 24 hr after a single oral dose of 1.3 mg/kg-bw. The elimination phase half-life from plasma is 2.7 hr and the urinary excretion half-life is 3.0 hr. These short half-lives resulted in virtually no residual melamine in tissues examined at 24 hr or later after exposure. The authors found little difference in levels in blood, liver and plasma, suggesting that melamine distributes in body water, with kidney levels slightly higher than plasma and liver levels. In the rat melamine is not metabolized (Mast *et al.*, 1983).
Buur *et al.* (2008) used the experimental data of Mast *et al.* to calibrate a 4-compartment rat and pig PBPK model for oral and intravenous administration (plasma, liver, kidney, carcass; absorption fraction: 100%) to describe the 24-hr. time-course of melamine after the oral dose mentioned above. Distribution in the body occurs by plasma transport. Melamine is eliminated from the body by renal clearance.

The rat PBPK model was scaled to pigs by Buur *et al*. (2008) and used to describe the time-course of melamine levels in this species as occurring after an intravenous injection of 6.13 mg/kg (Baynes *et al.*, 2008). Scaling from rat to pigs was done by adapting animal physiological model parameters (body weight, organ weights, cardiac output, organ blood flow, gastric emptying rate (Guerin *et al*., 2001) while maintaining the remaining model parameters at the values applied for rats (organ vascular space, partition coefficients, rate constant for absorption from the gastro-intestinal tract, renal clearance).

PBPK simulation of melamine transfer in pigs

Figure 1 shows the performance of the PBPK model of Buur *et al.* (2008) using the experimental data on the *i.v.* administration of melamine in pigs (Baynes *et al.* (2008). For the purpose of the current modelling approach, the model from Buur *et al*. was slightly modified by considering clearance of free concentration from kidney (RIVM model) instead of total concentration (model Buur *et al.,* 2008). This modification was deemed necessary, because the model as published by Buur *et al.* (2008) did not adequately describe the available data (see figure 3a in Buur *et al.*; 2008).

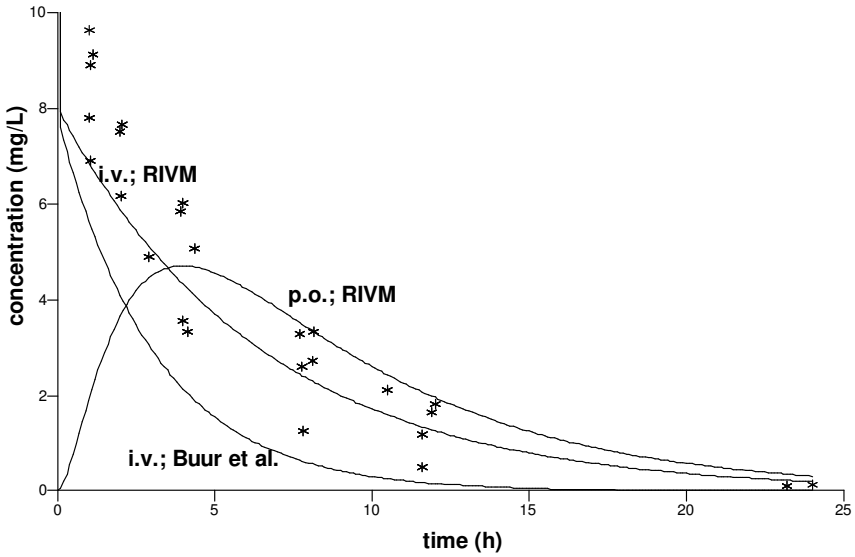

Figure 1. PBPK simulation of the time-course of the melamine concentration in plasma in pigs (body weight: 100 kg) given a single i.v. dose of 6.13 mg/kg body weight. **i.v.;Buur** *et al.* is the original model as described by Buur *et al.* (2008). **i.v.;RIVM** model is the Buur model but with adaptation for clearance of free melamine, instead of total melamine, *i.e.* free and bound. **p.o.;RIVM** is oral dose regimen as for iv model after expansion of the model with a module taken from Buur *et al.* (2008) to describe melamine absorption kinetics from the gastro-intestinal tract. Data (symbols): obtained after *i.v.* administration of 6.13 mg melamine /kg bw to pigs (Baynes *et al*., 2008)..

Figure 2 shows the concentration in plasma of swine (body weight 100 kg) after repeated oral dosing to 5.12 mg/kg twice a day (to facilitate comparison of the RIVM model outcome with the model predictions from Buur *et al.* (2008), who used the same dose regimen). The systemic availability is assumed to be 100%. The concentration ranges between 2000 and 5000 µg/L. Twenty four hours after the last dosing, plasma concentration has decreased to 300 µg/L. Figure 3 shows the corresponding concentrations in liver (range 3000 – 7700 µg/L) and muscle (assumed to contain melamine in its water phase (fraction 70%) only; range 1500 – 3500 µg/L). Twenty four hours after the last dosing, these concentrations have decreased to 500 and 230 µg/L, respectively.

In the PBPK model the kidney has a 2.4-fold higher affinity for melamine than the plasma. The reason for this may be that, as indicated by Mast *et al.* (1983), kidneys may contain urine which may contain a much higher melamine concentration than the kidney tissue itself. Hence kidney measurements used for model calibration for kidney tissue may be confounded by the presence of urine.

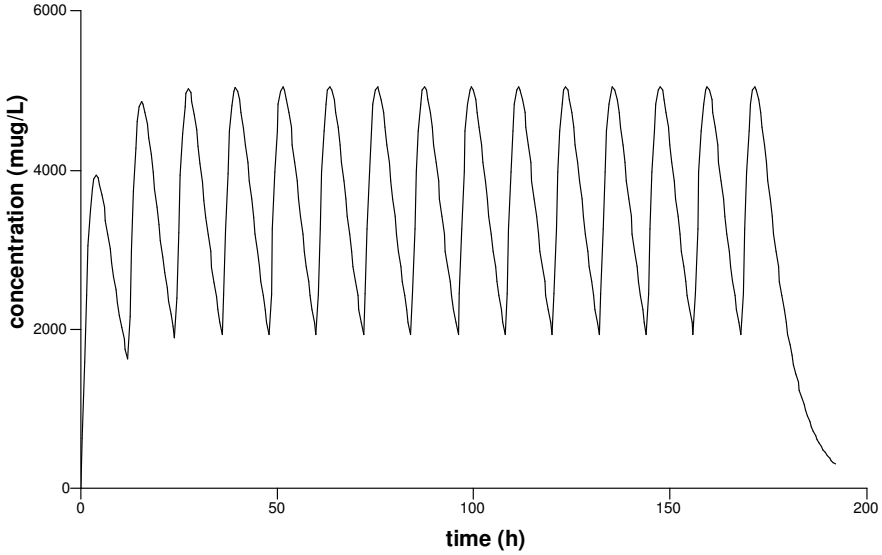

Figure 2. PBPK simulation the time-course of the concentration of melamine in the plasma of pigs after repeated oral dosing of 5.12 mg/kg twice a day for a total of 7 days (p.o. model as in Figure 1, dosing schedule adapted from Buur *et al.*, 2008).

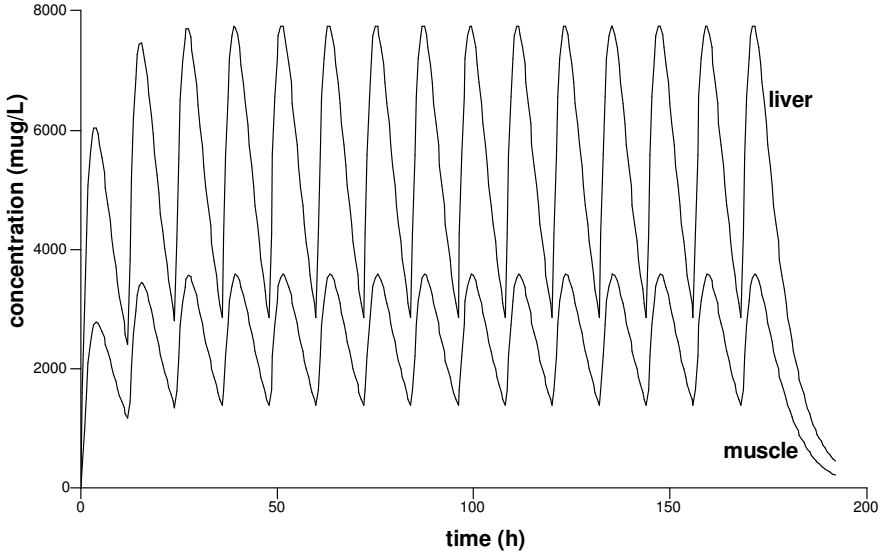

Figure 3. PBPK simulation the time-course of the concentration of melamine in the liver and muscle of pigs after repeated oral dosing of 5.12 mg/kg twice a day for a total of 7 days (p.o model as in Figure 1, dosing schedule adapted from Buur *et al.*, 2008).

Transfer of melamine after twice daily intake of 1 kg feed containing 500 mg melamine per kg feed (body weight: 40 kg)

The porcine PBPK model with adaptation of clearance from free kidney concentration was applied to a case where animals of 40 kg body weight are exposed to feed contaminated with 500 mg melamine per kg feed, fed in portions of one kg twice a day (i.e. in total 1000 mg/animal/day). This regimen implies intake of 12.5 mg melamine per kg body weight twice a day. To assess sensitivity to the model parameters, the same exposure was assumed to be administrated *i.v.* The model simulation of the concentrations in muscle are shown in Figure 4. Minimum and maximum concentrations after reaching plateau (in this situation a plateau is reached because the minimal and maximal concentrations are constant after ca 25 hours) are presented in Table 1.

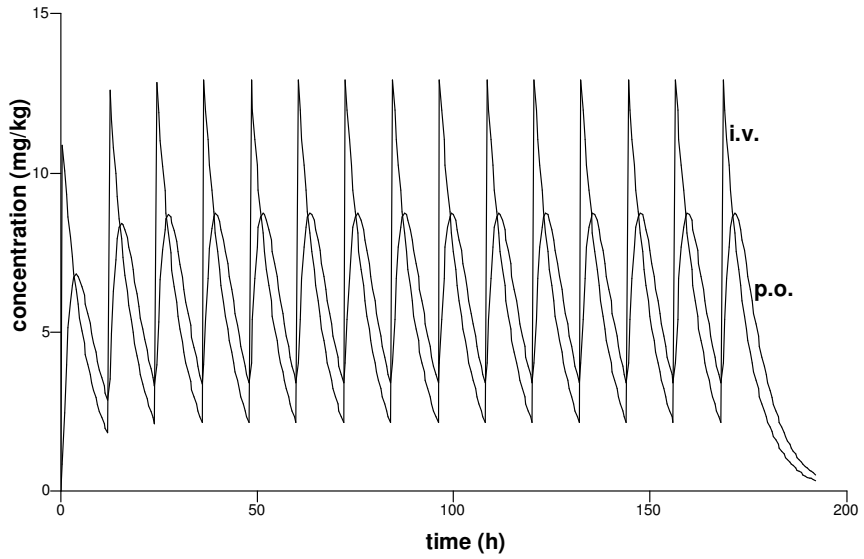

Figure 4. PBPK simulation of the time-course of melamine in muscle tissue of to pigs (40 kg) exposed to 500 mg/kg melamine in feed (i.e. 12.5 mg melamine/kg body weight) twice a day or intravenously, during seven days.

Table 1. Concentrations (C, in mg/kg; left sub columns) and transfer factors (TF, %; right sub columns) of melamine after reaching plateau.

	muscle		**liver**		**kidney**[a]		**plasma**[b]	
	C	TF	C	TF	C	TF	C	TF
minimum	3.5	0.7	7.2	1.6	10.8	2.2	4.9	1.0
maximum *p.o.*	8.7	1.7	18.9	3.8	26.8	5.4	12.3	2.5

a: results considered uncertain because of renal concentration of primary urine.

b: calculations are based on the well stirred approach. Peak plasma concentrations immediately after *i.v.* dosing, (i.e. when concentrations are at maximum) are unrealistic, because immediately after *i.v.* injection the substance is not homogenously distributed, yet.

Conclusion

The PBPK model as published by Buur *et al*. (2008) can well be used to simulate the transfer of melamine to plasma, liver and muscle of pigs exposed to this compound via feed. However, it

appeared that calculation including renal clearance based on free plasma melamine concentration rather than total plasma concentration was needed to describe the available experimental porcine data. The fact that this modified PBPK model was obtained from interspecies scaling from the rat to pigs may suggest that it could be applicable beyond these two species.

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References

Bayes, R.E., Smith, G., Sharon, E.M., Barrett, E., Barlow, B.M. and J.E. Riviere; Pharmacokinetics of melamine in pigs following intravenous administration; *Food Chem. Toxicol.* **46** (2008) 1196 – 1200

Buur, J.L., Baynes, R.E. and J.E. Riviere; Estimating meat withdrawal times in pigs exposed to melamine contaminated feed using a physiologically based pharmacokinetic model; *Regul. Toxicol. Pharmacol.* **51** (2008) 324 - 331

Mast, R.W., Jeffcoat, A.R., Sadler, B.M., Kraska, R.C. and M.A. Friedman; Metabolism, disposition and excretion of [^{14}C]-Melamine in male Fischer 344 rats; *Food Chem. Toxicol.* **21** (1983) 807 – 810.

Guerin, S., Ramonet, Y., LeCloarec, J., Meunier-Salaun, M.C., Bourguet, P. and C.H. Malbert; Changes in intragastric meal distribution are better predictors of gastric emptying rate in concious pigs than are meal viscosity or dietary fibre concentration; *Br. J. Nutr.* **85** (2001) 343 - 350