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## Original article:

# PRIMARY AROMATIC AMINES: THE CONTRIBUTION OF SEDIMENT ORGANISMS TO HUMAN EXPOSURE

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## ABSTRACT

3,4-Dichloroaniline (DCA) showed an unusually high bioconcentration factor (BCF) up to 800 in the sediment dweller *Lumbriculus variegatus*, exceeding BCFs found in fish and other aquatic organisms by a factor of 8–40. In the scope of the European Risk Assessment process for different aromatic amines, concern was expressed with regards to biomagnification and secondary poisoning of man. Although measured BCF data in fish may be not critical, biomagnification in food chain of sediment, sediment dwelling organisms, fish eating birds or mammal (including man) can not be excluded. To address this issue, the concentration of DCA in fish due to dietary uptake via *L. variegatus* (i. e., the biomagnifications factor, BMF) was calculated, based on two different assumptions: 1) Either DCA is accumulated in *L. variegatus* in the form of a metabolite, but instantaneously released as DCA in fish, or 2) a DCA metabolite is accumulated in *L. variegatus* and further accumulated in fish. In the first case, application of an existing experimental kinetic model showed that the experimental BCF is likely to increase from 22 to approx. 25 if uptake via food has to be taken into account. In the second case, use of a physiology-based toxicokinetic model (PBTK) resulted in a BCF of 1.7 to 46 for the DCA metabolite. The daily uptake for a consumer, given in the European Union Risk Assessment Report for DCA, would rise by 0.6 to 1.3 %. These analyses demonstrate that biomagnifications via sediment organisms is an exposure route that deserves attention in environmental risk assessments. However, the bioconcentration factor established in sediment organisms may overestimate the threat for human beings. The use of PBTK modeling is proposed as a means of estimating the increased daily uptake for a consumer.

**Keywords: sediment, aromatic amines, PBTK modeling, biomagnification**

## INTRODUCTION

For chemicals which have potential to be released into the environment, the issue of accumulation in the food chain as a possible route for secondary poisoning in man is one way of consumer exposure and, therefore, part of the risk assessment process in the European Union. The bioconcentration factor (BCF), measured in single-species tests with fish, is the classical data point to estimate the potential uptake of a chemical by man via the food chain. The OECD Guideline 305, which is most commonly used to measure BCF (OECD, 1996) does not consider accumulation via contaminated food (OECD, 1996). When the BCF for 3,4-dichloroaniline (DCA) was checked in several species, it turned out that the sediment dweller *Lumbriculus variegatus* showed an unusual high BCF of 800, which exceeded that of other aquatic species by a factor of 8–40 (Nagel, 1997). In the European Risk Assessment Report on

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DCA, it is mentioned that primary aromatic amines can form covalent bonds to humic matter in sediments. This results in a higher organic carbon-water partition-coefficient ($K_{OC}$) than anticipated from the octanol-water partition-coefficient ($K_{OW}$); this might explain the high BCF found for DCA in a sediment dweller (European Chemicals Bureau, 2006a). Therefore, accumulation in the sediment and biomagnification via the sediment dweller-fish-man food chain was identified as a reason for concern. A similar conclusion was drawn for 4,4'-methylene-dianiline (European Chemicals Bureau, 2001) and toluene-2,4-diamine (European Chemicals Bureau, 2006b) as well.

As the BCF of DCA in Lumbriculus seems to be critical with regards to consumer exposure on a first glance, a more detailed analysis is provided in this work to allow an estimation in how far the daily uptake for man is increased by the dietary route via the sediment. From experiments with *Lumbriculus variegatus* it could be derived that not 3,4-dichloroaniline (DCA) itself, but a metabolite was responsible for the high bioconcentration factor (Nagel, 1997). The metabolite was not identified. Without any prejudice, this metabolite is hypothesized to be a reaction product between DCA and humic matter, abbreviated here as HDCA. If this hypothetical molecule, HDCA, is taken up by trout, it might be either stable or might be cleaved rapidly to DCA. Both pathways shall be modeled in this document.

Whether the adduct between an aromatic amine and humic matter may easily liberate the parent amine in organisms, and whether the adduct exerts a higher or lower toxicological risk in terms of intrinsic toxicity and exposure, may be dealt with separately.

## MATERIALS AND METHODS

### *Kinetic model*

If the DCA metabolite, HDCA, is rapidly cleaved to DCA in trout, an existing kinetic model (Ensenbach et al., 1996) is extended to cover dietary uptake:

$$dC_F / dt = k_{01}' * C_W + k_d * C_F - 0.5 * C_F (k_a + k_b);$$

Steady-state assumption ($dC_F / dt = 0$):

$$BCF = C_F / C_W = k_{01}' / \{0.5 * (k_a + k_b) - k_d\}. \quad (1)$$

$C_F$: concentration in fish;
$C_W$: concentration in water;
$k_{01}'$: adsorption rate constant (~17.0 $h^{-1}$ for trout);
$k_a$: elimination rate constant for compartment A (1.48 $h^{-1}$ for trout);
$k_b$: elimination rate constant for compartment B (0.03 $h^{-1}$ for trout);
$k_d$: dietary uptake rate constant ($h^{-1}$).

$k_d$ is the result from the amount of food taken up per day, the concentration of HDCA in Lumbriculus (which is 40 times that of DCA in trout), and resorption efficiency.

### *PBTK model*

If HDCA is not cleaved to DCA rapidly, the distribution and accumulation of HDCA in trout can be calculated by physiology based toxicokinetic modeling. Here, a model of Nichols (Nichols et al., 2004, 2007) is applied and slightly modified. Figure 1 is a visualization of the model. Physiological parameters for trout were taken from Nichols et al. (2004). The log $P_{OW}$ of HDCA was calculated as 4.2, based on the BCF of 800 in *Lumbriculus variegatus* and the linear relationship given in the European Technical Guidance Document on Risk Assessment (European Chemicals Bureau, 2003a):

$$\log BCF = 0.85 * \log K_{OW} - 0.7.$$

The blood-water ($P_{bw}$) and tissue-blood ($P_{tb}$) partition coefficients were calculated based on algorithms published by Bertelsen (Bertelsen et al., 1998). Physiological data

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and constants for the hypothetical metabolite HDCA are given in the appendix. Trout has a daily food-uptake of 0.5–4.0 % of body weight (Nichols et al., 2004; Eimer, 2006). DCA, which reacted with organic matter, is not easily resorbed in the gut (Sandermann et al., 1992). As these authors show, the resorption efficiency of "insoluble" DCA-metabolites in sheep and rats was approx. 15 %. Therefore, a resorption efficiency of 25 % may already be regarded as a worst case. If reference is made to pentachlorobiphenyl, the fugacity in the chymus in the gut is 4 times higher than that in the food (Gobas et al., 1999). This would mean a resorption efficiency of 75 %. For a trout of 1 kg weight, the estimated internal uptake of HDCA *via* Lumbriculus ingestion is:

Dietary resorption:
0.001 – 0.004 µg/min = 0.06 – 0.24 µg/h.

As HDCA is a hypothetical molecule, the renal clearance, CLr, is difficult to estimate. The renal elimination rate established for pyrene, 1.049 * $10^{-4}$ L/min (Law et al., 1991), is taken as a default.

Concerning hepatic metabolism, the parameters for the Michaelis-Menten equation, $V_{max}$ and $K_m$, were assumed to be between 0 and 5.8 µg/min and 3050 µg/L, respectively. The data are those from pyrene, as well (Law et al., 1991). With these assumptions the whole range from no to extensive metabolism seems to be covered, as can be deduced from data for hepatic clearance in fish summarized by Han (Han et al., 2007). To convert pmol/min/g to µg/min for $V_{max}$, a trout of 1 kg weight was assumed to have a liver weight of 13 g and approx. 35 mg microsomal protein per g liver (Nichols et al., 2006). A best match between model and measured data is obtained if the substance in blood is assumed to be 100 % available for metabolic transformation (Nichols et al., 2007).

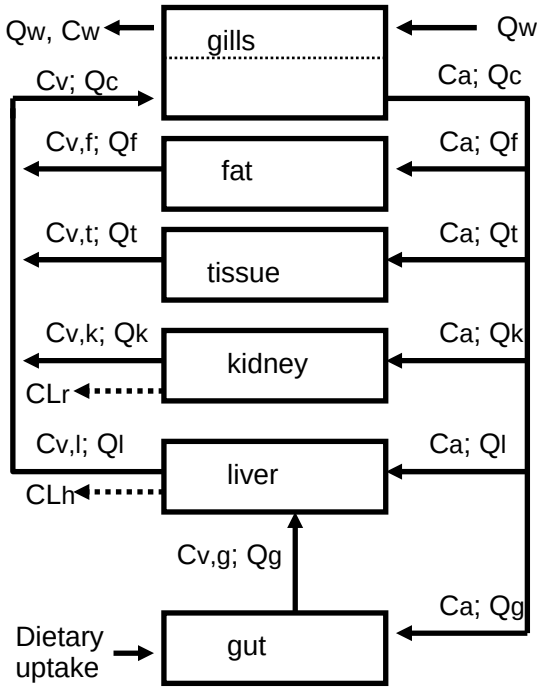


**Figure 1:** PBTK-model for HDCA, based on a PBPK-model by Nichols et al. (2004), with modifications. Poorly perfused tissue was omitted. As infinite dilution in water is assumed, HDCA is excreted, but not taken up by the gills. Q: flow of blood or water, respectively; $C_a$: concentration in arteria, leaving the gills; $C_v$: venous concentration CLr: renal clearance; CLh: hepatic clearance.

As only *Lumbriculus variegatus* showed an unusually high BCF for DCA (Nagel, 1997) it is assumed, that the concentration of the critical metabolite in water is negligible. That means that excretion *via* the gills may be an important route for depuration in trout. This excretion may either be modeled by an equilibrium partitioning, where the establishment of an equilibrium of HDCA between blood and water within a short period of time is assumed; alternatively, a diffusion-controlled kinetic model is assumed.

*Excretion via gills*

Taking the equilibrium model, based on the blood-water partition coefficient ($P_{bw}$), blood leaving the gills would have a HDCA concentration of 94 % of the blood entering the gills.

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For the diffusive model, use is made of Fick's first law of diffusion:

$dn/dt = D * \delta C * A/L,$

D: diffusion coefficient (m²/s);
δC: gradient of concentration over the intersphere;
A: contact surface (m²);
L: thickness of boundary layer (m).

D can be calculated with the Reddy-Doraiswamy equation (Reddy and Doraiswamy, 1967):

$$D_{AB} = 10^{-11} * M_A^{0.5} * T / (\acute{\eta}_B * (v_A * v_B)^{1/3})$$

$$= 2.69 * 10^{-9} \text{ m}^2/\text{s}.$$

$M_A$: Molecular weight of the HDCA, 700 g/mol assumed.
T: Temperature, 10 °C.
$\acute{\eta}_B$: Viscosity of water at temperature T (10 °C = 285 K), = 1.2028 cP.
v: molar volumes; 18 cm³ for water, 700 cm³ for HDCA assumed.

The assumed molecular weight of 700 g/mol for HDCA is a kind of worst case concerning diffusivity. Molecules with a higher molecular weight are generally thought to pass less easily biological membranes (European Chemicals Bureau, 2003a). As proposed by Erickson and McKim (1990), the diffusion across the gill membranes is assumed as being 50 % of the diffusivity in water. For trout of 1 kg weight, A = 0.15 m² and L = 1.5 * $10^{-4}$ m (Nichols et al., 2004). Taking these parameters, and converting the dimensions accordingly, Fick's equation reads:

$dn/dt = \sim 0.081 \text{ L/min} * \delta C$

which is the amount of HDCA per minute that is excreted *via* gills into the water. The amount of HDCA entering the gills with the venous blood has to be the same as the amount leaving the gills in water and arterial blood; that is

$$Q_c * C_v = Q_c * C_a - 0.081 \text{ L/min} * \delta C,$$

$C_a$: Concentration in arterial blood;
$C_v$: Concentration in venous blood;
$Q_c$: Cardiac output, passing the gills: 0.035 L/min.

Now, δC is a function of $C_w$, the concentration that can be achieved once equilibrium is established, *i. e.* $C_w = C_a / P_{bw}$. This assumption is justified as fish gills are operating in a counter-current mode. This means, that the concentration of HDCA in blood leaving the gills is 97.9 % of the blood concentration entering the gills.

*Software*

The PBTK model was run with Microsoft Excel®, as described by Haddad (Haddad et al., 1996). The time intervals chosen were one minute.

*Calculation of dietary uptake by man*

The dietary uptake of HDCA / DCA by consumers eating trout was calculated with an equation given in the Technical Guidance Document on Risk Assessment (European Chemicals Bureau, 2003b):

Daily dose (µg/kg/d) =
$C_{fish}$ * daily uptake fish / body weight =
$C_{fish}$ (µg/kg) * 0.115 (kg / d) / 70 kg. (**2**)

## RESULTS

*HDCA suffers rapid cleavage to DCA in trout*

In case the DCA metabolite is rapidly cleaved to DCA in trout, equation **1** may be applied to calculate the BCF of DCA in trout. In *Lumbriculus variegatus*, the concentration of DCA is ~ 40 times higher than in trout (BCF of 800 against 22). The additional maximum uptake rate due to this monotone food source (40 g Lumbriculi per day for 1 kg trout) would be:

$40 * C_{fish} / 24h * 0.04 = 0.0668 * C_{fish} / h.$

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This is the highest, theoretically possible value. However, taking a more realistic uptake into consideration (daily food uptake is at maximum 0.01 * body weight) and 75 % resorption in the gut, the additional uptake would be:

$40 * C_{fish} / 24h * 0.01 * 0.75 =$
$0.0125 * C_{fish} / h.$

As a result, $k_d$ in equation **1** has the value 0.0125–0.0668 $h^{-1}$, and the BCF increases from 22 to 22.9–24.7. Assuming a concentration of 0.7 µg/L for DCA in the water (European Chemicals Bureau, 2006a), trout would contain 16.0–17.3 µg/kg DCA. The daily intake for man, according to equation **2**, would be 26.3–28.4 µg/kg BW/d. Taking the data of the European Risk Assessment Report on DCA (European Chemicals Bureau, 2006a), the daily intake for a consumer would increase from 4.022 µg/kg BW/d to 4.026–4.028 µg/kg BW/d, which is an increase by 0.1–0.15 %.

*HDCA is not rapidly cleaved to DCA in trout*

In this case, the PBTK model outlined above is applied. For the excretion via gills the diffusion model is applied which yields higher tissue concentrations in fish than the equilibrium model. The concentration of HDCA in muscle is slightly less than 2 % of the HDCA concentration in fat (data not shown). Therefore, in the following charts only the concentration in fat is shown. Trout is estimated to consist of 10 % fat tissue, 20 % non-fat tissue (muscle/general tissue) and 70 % of water (Nichols et al., 2004). To calculate the concentration of HDCA in trout, the composition is assumed to be 10 % fat tissue and 90 % non-fat tissue, which will slightly overestimate the total concentration in trout. That is,

$$C_{fish} = 0.1 * C_{fat} + 0.9 * C_{non\text{-}fat} \Leftrightarrow$$
$$C_{fish} = 0.1 * C_{fat} + 0.9 * 0.02 * C_{fat} \qquad (3)$$

How food intake influences the HDCA concentration in fat tissue is shown in figure 2.

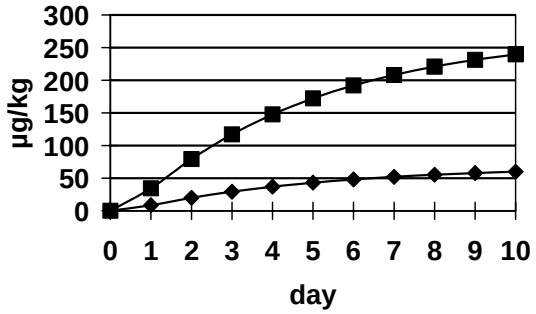


**Figure 2:** HDCA concentration in fish fat tissue. Hepatic clearance is considered not to take place. Squares: uptake 0.004 µg/min; diamonds: uptake 0.001 µg/min

Metabolic transformation has an influence on the equilibrium concentration of HDCA in trout. If a comparatively low $V_{max}$ is linked to a high Michaelis-Menten constant, $K_m$, the effect of metabolism on the equilibrium concentration of HDCA is small to negligible (data not shown). However, if data gained with pyrene are used as a surrogate (Law et al., 1991), an approximately 5-fold lower concentration of HDCA in fat tissue against no-hepatic clearance is the result (figure 3). From figures 2 and 3, the final concentration of HDCA in trout can be estimated.

Use of equations 3 and 2 allows a calculation of the concentration of HDCA in trout, and the daily uptake by man, respectively.

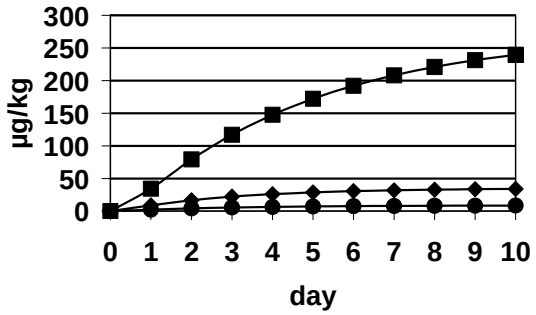


**Figure 3:** HDCA-concentration in fat of trout, depending on hepatic metabolism and dietary uptake. Squares: uptake 0.004 µg/min, $V_{max}$ = 0; diamonds: uptake 0.004 µg/min, $V_{max}$ = 5.8 µg/min, $K_m$ = 3050 µg/L; circles: uptake 0.001 µg/min, $V_{max}$ = 5.8 µg/min, $K_m$ = 3050 µg/L.

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**Table 1:** Daily human dose due to uptake of HDCA via fish, in dependence of dietary uptake of HDCA by trout and hepatic clearance

| Uptake by trout | $V_{max}$, $K_m$ | $C_{fish, fat}$ | $C_{fish}$ | Daily dose |
|---|---|---|---|---|
| 0.004 µg/min | 0, $10^5$ | ~ 275 µg/kg | 32.45 µg/kg | 0.0533 µg/kg BW |
| 0.004 µg/min | 5.8 µg/min, 3050 µg/L | ~ 40 µg/kg | 4.72 µg/kg | 0.0078 µg/kg BW |
| 0.001 µg/min | 0, $10^5$ | ~ 70 µg/kg | 8.26 µg/kg | 0.0136 µg/kg BW |
| 0.001 µg/min | 5.8 µg/min, 3050 µg/L | ~ 10 µg/kg | 1.18 µg/kg | 0.0019 µg/kg BW |

With a DCA-concentration in the water of 0.7 µg/L (European Chemicals Bureau, 2006a), the final concentration of HDCA in trout ranges from 1 to ~33 µg/kg (table 1). The BCF of HDCA is 1.7–46.4. If this BCF of HDCA is added to the BCF of DCA, the "total" BCF would range from 23.7–68.4. Due to HDCA-accumulation in trout, the daily intake of DCA + HDCA would increase from 4.022 µg/kg BW/d (European Chemicals Bureau, 2006a) to 4.024–4.075 µg/kg BW/d. This is an increase by 0.05–1.32 %.

## DISCUSSION

In a recent article, the incomplete picture of biomagnification in the food chain that is provided by flow-through tests with fish is highlighted with respect to terrestial and sea-mammal food chains (Kelly et al., 2007). To discover a high bioaccumulation at one stage in the food chain for a substance that was deemed not to be critical in terms of bioconcentration is alarming at a first glance. Although trout and Lumbriculus do not share a common habitat, modeling based on these organisms makes sense with respect to available data and possibility of testing. By using the PBTK model as proposed in this work, a step towards a quantification of the risk is possible. However, as the critical metabolite was not known, several imponderables are included in the model, mainly in terms of diffusivity, $P_{ow}$ and metabolism. Further, it was assumed that metabolism results in a disappearance of the substance. This is not necessarily equal to a disappearance of a risk. In fact, the bioaccumulation study with DCA in Lumbriculus has shown that not DCA itself, but some metabolite was accumulated (Nagel, 1997). In discussions about the bioaccumulation potential of a substance, it needs to be made clear whether the interest focusses on the individual substance or whether all metabolites are included. The modeling of the parent compound including metabolism actually ends up in a BCF related to this individual compound. A measured BCF in terms of radioactivity will end up in a higher value, as now all metabolites present in the tissues of the organism are included. If metabolism of a substance does not end up in an inert, non-mineral metabolite, the PBTK approach is regarded as acceptable, as the negligence of metabolites is probably sufficiently balanced by several worst-case assumptions: a) no metabolism or metabolism only in liver, although also other tissues of fish show metabolic capacity (Kleinow et al., 1998; Joensson et al., 2006); b) a worst case resorption efficiency for HDCA; c) a high daily intake of Lumbriculi by trout; d) a monotone food source; e) no growth-dilution; and f) no excretion via bile. In fact, if metabolism occurs, it is likely that the metabolites are more easily excreted as they are more polar than the parent compound. The base-line bioaccumulation model (OASIS CATABOL M, v5.100), described by Dimitrov et al. (2005) was used to illustrate this assertion. This model estimates maximum BCF values based on the multi-compartment partitioning model for passive diffusion, with correction for mitigating factors of three-dimensional molecular size, ionization (*i. e.*, acids and phenols), and potential for Phase I and Phase II biotransformations in fish liver. The poten-

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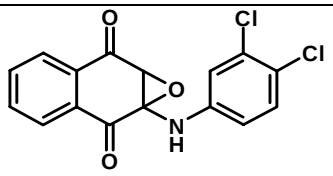
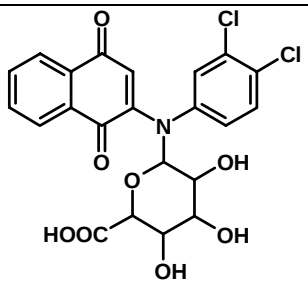
tial for metabolism, as well prediction of probable metabolites, is derived from a database of 382 Phase I and 48 Phase II transformations in mammalian (rat) liver. Model predictions were performed for a hypothetical compound for HDCA, 2-N(3,4-dichlorophenyl)naphtho-quinone-1,4.

The estimated probabilities for various metabolic transformations (P) of this substance, as well as predicted log $P_{ow}$ BCF values for the resulting metabolites, are summarized in Table 2.

**Table 2:** Probabilities of obtaining (P) and estimated log $K_{ow}$ and log BCF values for the hypothetical HDCA substance and its possible metabolites, as predicted using the OASIS CATABOL model. Not shown: DCA-ring hydroxylation products and mercapturic acid conjugates of the DCA-moiety, having a calculated probability of 0.00.

| Probability | Structure | Transformation | log $P_{ow}$ | log BCF |
|---|---|---|---|---|
| 1.00 | 2-N(3,4-dichloro-phenyl)naphthoquinone-1,4 | ---- | 4.6 | 2.5 |
| 0.34 |  | epoxidation | 5.7 | 0.6 |
| 0.29 |  | N-glucuronidation | 3.6 | 0.4 |

Note, that only the epoxide is prone to release DCA after hydrolysis of the epoxide-ring, which will result in an instable semi-aminal. As mentioned earlier, the question of the toxicity of reaction products of primary aromatic amines with humic matter and their metabolites is not dealt with here.

Depending on the metabolism of the critical DCA metabolite, the increase in biomagnification in trout due to ingestion of contaminated lumbriculi ranges from marginal to very important. This demonstrates the general need to take this way of biomagnification into account while performing environmental risk assessments with focus of secondary poisoning of consumers. If bioaccumulation tests in lumbriculus are performed, the log $P_{ow}$ and some information concerning the structure of the metabolites may be derived with some additional, not too extensive effort. These would help to reduce uncertainties in the modelling. In vitro metabolism studies with fish hepatocytes or liver S9 homogenates – if feasible – would reduce uncertainties concerning metabolism.

The above mentioned PBTK model was checked with DCA which has an experimental BCF of 22 in trout. In that case, gills were modeled as a compartment where equilibrium partitioning takes place as in all other tissues, and the concentration in water was set at constantly 0.7 µg/L. Dietary uptake was assumed to be zero. The model yielded a BCF of 40. This is not very different from 22; however, to achieve a BCF of 22, metabolism has to be assumed not only in the liver, but also in other tissues, and DCA has to suffer some clearance be-

fore it arrives at fat tissue. This shows that the PBTK model applied still is a crude simplification of the complexity of the living organism.

The advantage of PBTK-modeling is that in addition to the BCF, the time-course of the concentration in tissue and tissue distribution can be estimated.

Taking the results from the PBTK model for HDCA, the increased risk for secondary poisoning is small for DCA. The main reasons are other, more important pathways for uptake, *p. e.* 1.7 µg/kg BW/d for drinking water and 2.3 µg/kg BW/d for crop. Therefore, the increased daily intake due to the results of the PBTK modeling - 4.024–4.075 µg/kg BW/d against 4.022 µg/kg BW/d – still is well away from the lowest $NOAEL_{man}$ which is 750 µg/kg BW/d (European Chemicals Bureau, 2006a). For other primary aromatic amines, a case-to-case consideration is necessary, taking into consideration all possible pathways for intake. However, experience gained with DCA should be taken into account. For example, if data of the primary aromatic amine under consideration indicate already a faster metabolism of the non-nitrogen moiety than DCA, the same can be expected from adducts with humic matter. A scientific well thought through approach should be preferred against simple box-ticking.

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## APPENDIX

For the physiology based toxicokinetic modeling, the following parameters were used, based on published data (Nichols et al., 2004, 2006):

Effective respiratory volume, $Q_w$: 0.123 L/min; Cardiac output: 0.0035 L/min

Compartment volumes as fraction of body weight:

Fat volume $V_f$: 0.04
Liver volume $V_l$: 0.013
Tissue volume $V_t$: 0.89
Gut volume $V_g$ : 0.048
Kidney volume $V_k$ : 0.008
Blood-flow gills $Q_c$: 0.035 L/min
Blood-flow tissue $Q_t$: 0.0232 L/min
Blood flow fat $Q_f$: 0.00119 L/min
Blood flow liver $Q_l$: 0.00102 L/min
Portal vein flow $Q_{pv}$: 0.00708 L/min
Blood flow gut $Q_g$: 0.00708 L/min
Blood flow kidney $Q_k$:0.00196 L/min
Total flow ex liver $Q_{lv}$: 0.0081 L/min

Blood-water and tissue-water distribution coefficients, (Bertelsen et al., 1998):

$P_{tissue\text{-}water} = 10^{(a * \log P_{ow} + b * \log(\text{lipid fraction}) + c)} + \gamma$, and
$P_{tissue\text{-}blood} = P_{tissue\text{-}water} / P_{blood\text{-}water}$

Blood: a = 0.65; b = 1.72; log(lipid fraction) = -1.854; c = 2,49; $\gamma$ = 0.839; $P_{bw}$ = 108;
Fat: a = 0.9; b = 0.56; log(lipid fraction) = -0.026; c = 0.26; $\gamma$ = 0.05; $P_{fw}$ = 10603; $P_{fb}$ = 98.2;
Liver: a = 0.97; b = 2.17; log(lipid fraction) = -1.347; c = 0.746; $P_{lw}$ = 527; $P_{lb}$ = 4.88;
Muscle / general tissue: a = 0.69; b = 0.92; log(lipid fraction) = -1.523; c = 0.76; $\gamma$ = 0.769; $P_{mw}$ = 181; $P_{mb}$ = 1.68;
Kidney: a = 0.97; b = 2.17; log(lipid fraction) = -1.284; c = 1.57; $\gamma$ = 0.789; $P_{kw}$ = 721; $P_{kb}$ = 6.68;

Data for gut tissue were not available. It was assumed that gut tissue has the same properties as muscle/general tissue.

Mass-balance in tissue (fat, muscle):
$dC_t/dt * V_t = Q_t * (C_a - C_t / P_{tb})$;
Mass-balance gut-tissue:
$dC_g/dt * V_g = Q_g * (C_a - C_g / P_{gb})$ + dietary intake; dietary intake: 1 – 4 ng/min;
Mass-balance liver-tissue:
$dC_l/dt * V_l = Q_l * C_a + Q_g * (C_g / P_{gb}) - Q_{lv} * (C_l / P_{lb}) - (V_{max} * C_l / P_{lb}) / (K_m + C_l / P_{lb})$ ;
Mass-balance kidney:
$dC_k/dt * V_k = Q_k * (C_a - C_k / P_{kb})$ - CLr;
$CLr = 1.049 * 10^{-4}$ L/min $* C_k / P_{kb}$.