

Accumulation of organic chemicals by earthworms from food

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Earthworms form a major part of the soil biomass in many terrestrial systems and are important contributors to the diet of avian and mammalian species (a.o. badgers, hedgehogs, foxes, blackbirds, thrushes). Accumulation of organic contaminants (xenobiotics) in earthworms is therefore not only a threat to the earthworms themselves but also for the health of the organisms preying on them. The uptake of organic chemicals in soil- and sediment-dwelling organisms is usually described as the net result of the hydrophobic partitioning between soil organic matter, pore water, and the organism's lipids. This is generally called the equilibrium-partition approach (EP). EP is applied to predict body residues in organisms but also to derive quality criteria for soil and sediment from water criteria. The EP assumption is generally quite successful although several factors may limit its applicability. One of these factors is that organisms may additionally take up chemicals through feeding. Earthworms live in close contact with the soil and most species also consume large quantities of soil to digest the organic constituents and for the construction of burrows. Earthworms are able to take up chemicals from water through the outer skin as well as from their food but the exact quantitative contributions of each route are unclear. I am interested in how much organic (xenobiotic) chemicals are accumulated by earthworms from their food. This is interesting from the perspective of contaminated soils, but especially when the food of the worms is specifically contaminated. Think about litter in orchards after pesticide spraying and manure after the cattle have been treated for parasitic worms.

In many publications, accumulation from food is treated as a route that is fully additive to the results of uptake from the water phase. The DEB book follows this convention and assumes that the uptake across the gut wall is a constant fraction of the ingestion flux ($C_{\text{ingesta}}/C_{\text{egesta}}=\text{constant}$). This implies that when this fraction is not zero, the organism will reach a steady-state body residue that exceeds that expected by EP. Firstly, this approach is, in my opinion, an oversimplification of the actual dynamic process underlying uptake from food. This assimilated fraction is a compound parameter that depends on many physiological parameters already included in the DEB model (like retention time and volume of the gut contents). Estimating this fraction will therefore teach us little about the underlying processes. Secondly, the Equations 6.16/6.17 are not a correct simplification as elimination through the skin and through the gut contents must be treated separately (the uptake rate constant from water is related to k_{ew} , uptake from food to k_{eX} , elimination to $k_e=k_{ew}+k_{eX}$). A more detailed, first-principle, approach is proposed here. The gut contents are still "outside" of the animal, and there is no fundamental difference between uptake from the (pore) water phase over the

external skin (or gills) and over the gut wall. Frank Gobas and co-workers (1993a/b) demonstrated that the mechanism of gastrointestinal uptake in fish is simple passive diffusion from the dissolved phase in the gastrointestinal tract (GIT) to the organism's tissue. The main differences between the two routes is that the gut surface is large, excretions from the organism are present (e.g. digestive enzymes), and the volume is limited (compared to the outside world). Even though gut surface is proportional to the outer skin surface (for isomorphs), the limited volume of the gut contents may force us to add the gut as a separate compartment.

Suppose we have an earthworm that consumes soil. If the earthworm does not affect the chemical availability (or fugacity) in the gut contents, there is no additional uptake through this route. When a clean worm is placed in soil, initially, the concentration gradient from soil water (in the gut and on the outside) to the worm is large and uptake will result. Since the gut surface probably exceeds the outer surface, the main uptake will be from the gut contents. In this situation, the concentration in the egesta will be (much) lower than in ingesta. However, as the earthworm approaches steady state, the concentration gradient, and thus the uptake flux, decreases. Chemicals are still taken up from the gut contents, but the return flow (from worm tissues to gut contents) is equally large. In this situation, the fraction taken up from the ingestion flux becomes zero. This process can, however, lead to higher body residues than predicted by EP because the availability of the chemicals is affected by gut passage. Gut passage increases the fugacity of the chemical because the sorption capacity of the food is decreased (digestion of sorption sites: lipids and organic matter), and because its contents may be compacted (food absorption). Of course, the food changes in more ways during gut passage (e.g. in pH), but these changes do not generally influence bioavailability of neutral organic compounds. Whether feeding actually leads to higher body residues depends on the magnitude of the fugacity increase, and the magnitude of the elimination rate with the faeces compared to the other elimination routes. If elimination across the skin (or gills in fish) is fast, there will be a net flux from gut contents to outside world but it will not lead to higher body residues than expected, based on EP.

A model for earthworms based on these principles has been developed (Tjalling Jager, submitted), and calibrated to existing accumulation data. The problem is that the physiological parameters of earthworms are not well known, and especially not in relation to accumulation of toxicants. The parameters that need to be quantified are the volume of the gut (M_{gm}), gut retention time (t_g) or ingestion rate (J_X), and the digestion of sorption sites. Furthermore, the earthworms may select for patches of soils that are particularly rich in organic matter or micro-organisms. Preliminary results show that the compost worm (*Eisenia andrei*) is able to select material with the twice the amount of

organic carbon of bulk soil when feeding on OECD artificial soil. Compaction seems to be negligible in most situations but may be added to the model.

The DEB model provides handles to deal with these parameters. The ingestion rate is given at {75} as $J_X = \{J_{Xm}\}fV^{2/3}$, which implies that the feeding rate is taken proportional to a surface area. The proportionality constant is assumed to depend on the composition of the diet. The gut residence time on {81} is taken as $t_g = [M_{gm}]V/J_X$. The maximum gut volume is taken proportional to body volume, which seems to correspond to the data for earthworms (Hartenstein *et al.*, 1981; Bolton & Phillipson, 1976), as long as the diet is kept constant. Since J_X is surface-area dependent and M_{gm} volume dependent, the gut residence time, t_g , is proportional to length. This finding is not confirmed for earthworms. Feeding rates and residence times are very difficult to determine experimentally but, in general, the gut residence time seems to be independent of size (Hartenstein *et al.*, 1981; Bolton & Phillipson, 1976). Only for the species *Aporrectodea rosea*, a relation between size and t_g was observed when the worms were kept at a relatively high temperature of 15°C (Bolton & Phillipsons, 1976)

DEB takes the assimilation efficiency of food independent of the feeding rate, and thus, the assimilation rate proportional to the ingestion rate: $\{p_{Am}\} = \mu_{AX}\{J_{Xm}\}$ where μ_{AX} is the conversion efficiency of food in assimilated energy. If t_g is independent of length, this can only be reconciled with an ingestion rate being proportional to volume instead of surface area. This was indeed observed for *A. rosea* when this species was kept at temperatures of 4.4 and 10°C. The egestion rate (and therefore also the ingestion rate) is exactly proportional to body weight. At 15°C, the smaller individuals are able to increase their volume-specific ingestion rate, but the larger ones cannot. When the ingestion rate is indeed proportional to a volume, this would have the weird consequence that p_{Am} will be proportional to volume also, which implies that there is no maximum size of the earthworm (because maintenance is also proportional to body volume). This conclusion, however, does not agree with what we know from earthworms by experience. We can obtain a maximum body size when we assume that μ_{AX} is not constant but is, to some extent, negatively related to body volume. If t_g is constant with size, this means that the speed at which the food passes a cm of gut wall is larger in a large worm than in a small worm. If we take μ_{AX} inversely proportional to the length of the gut ($\mu_{AX} \propto V^{-1/3}$) and ingestion rate proportional to volume ($J_{Xm} \propto V$) we obtain that p_{Am} is still proportional to a surface area (since $p_{Am} = \mu_{AX} J_{Xm}$). This is in principle a testable result; small individuals should have higher assimilation efficiencies than large ones. Perhaps the easily measurable carbon content (or C/N ratio) in ingesta and faeces can be related to the energy gain by the earthworm.

DEB states that μ_{AX} and $\{J_{Xm}\}$ depend on the composition of the diet, but not *how* they depend on the diet. Furthermore, it is clear that earthworms also vary their gut volume M_g (or at least the weight of the gut contents) with diet composition (Hartenstein *et al.*, 1981; Hendriksen, 1991). On a rich diet (sludge or manure), the weight of the gut contents is lower than on poor food (soil only). In fact, gut load could be related quite nicely to C/N ratio in the food. If we assume that gut retention time is independent of food quality (which seems reasonable), it follows that the ingestion rate drops with increasing food quality. In other words, an increase in μ_{AX} would be accompanied by a decrease of J_{Xm} . It is possible that these processes cancel out which would lead to a $\{p_{Am}\}$ that is largely independent of food quality.

Earthworms are generally quite flexible in their response when food abundance varies. A species like *Eisenia andrei* is able to survive low-food situations by prolonging (or reverting back to) its juvenile state. However, if the argument presented is valid, it seems that earthworms are to some extent demand systems. These organisms seem to be able to adapt their feeding behaviour to keep the flow of assimilated energy approximately constant. This has consequences for accumulation of chemicals through feeding. The uptake rate constant from the gut contents is determined directly by μ_{AX} as a high conversion leads to more digestion of sorption sites and thus an increase in fugacity. On the other hand, high quality food with a high μ_{AX} leads to a low gut load, which may be rapidly depleted. In the latter case, the retention time may be too slow to sustain a large uptake flux from the food source. In summary, the link between feeding and accumulation may be worked out much further than described on {197}. Furthermore, a one-compartment approach will be insufficient to describe short-term experiments for bioaccumulation, or rapidly changing food sources or changes in chemical content. For these situations, a separate dynamic compartment for the gut contents is required. However, proper experiments to test the proposed model formulation have not been performed so far. What is needed are accumulation experiments (with and without contaminated food) in which also the physiological parameters related to feeding are determined: gut retention time, gut volume, digestion efficiency, and sorption in the gut contents.

References:

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