Carry-over of diethylhexylphthalate and aromatic nitro compounds into the milk of lactating cows

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1 Introduction and scope

The role of milk fat as an accumulating substrate for an abundance of lipophilic pollutants and residues is well documented for a considerable number of anthropogenic and non-anthropogenic chemicals. There was, however, a lack of information about the secretory carry-over of phthalic acid esters, probably the most widespread environmental chemicals world-wide, into milk fat as compared to the multiple post-secretory routes of milk contamination, beginning with the rubber parts of the milking machine. The role of the nitro musk compounds, widely used surrogates and/or fixatives for natural musk oils, is apparently of marginal importance in dairying. Nitro musk compounds show a marked bio- and geoaccumulation, are fat-soluble and can be found in freshwater fish in concentrations resembling those of the higher chlorinated biphenyl congeners. The polynitro aromatics could play a role in milk contamination, when pastures and other forage cultures are either occasionally flooded by annual floodings or regularly irrigated with water from rivers or from filter beds.

The prevalence of the phthalic acid ester diethylhexylphthalate – DEHP – in milk is regularly and originates from an abundance of possible sources, though not to be clearly identifiable due to a high background contamination. Nothing has been reported in literature about the prevalence of nitro musk compounds – musk ambrette, musk tibetene, musk xylene, musk ketone, musk moskene – in cows milk. Their findings in human substrates result to a large extent from the dermal absorption after the use of perfumes and/or (washed) clothing (RIMKUS & WOLF 1993, KOKOT-HELBLING *et al.* 1995.

The intention of the studies reported here was to quantify the secretory carry-over

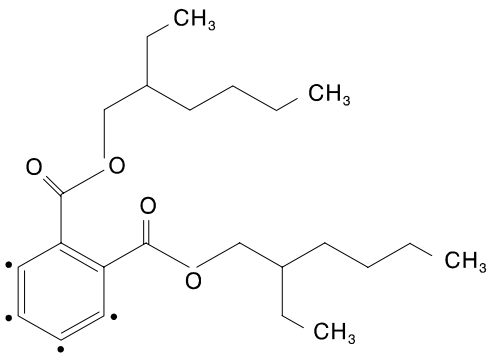
– of DEHP in the milk of lactating cows after oral supplementation as compared to the difference in the DEHP content in hand-milked and machine-milked milk and

– of the five nitro musk compounds to get information over a possible contaminatory pathway to the consumer from the terrestric food chain and to derive possible similarities in the food chain behaviour of other polynitro aromatics from military explosives in polluted areas which are under recultivation.

2 Materials and methods

2.1 Diethylhexylphthalate

To overcome the considerable difficulties of a widespread and hardly to be excluded background contamination with DEHP in practically all substrates, chemicals and equipment involved, we used the tetradeuterated specimen as given in the first figure:



Phthalic acid-bis-2-ethylhexylester, D_4 -(DEHP)
$C_{24}D_4H_{34}O_4$ M_r = 394.57
5_016/97 nb

Figure 1

The use of deuterated DEHP in combination with the final determination by GC/MS excludes every unspecific background contamination completely and gives true figures for the oral carry-over.

The substance was dissolved in acetone and dosed to two lactating cows in doses of 100 and 300 mg/d, respectively, via gelatine capsules filled with starch as a carrier for the dose. The supplementation period was 10 days, the analytics in milk as outlined below were carried out in real time.

Analysis of phthalic acid-bis-2-ethylhexylester (DEHP D_4) in milk	
– **Sampling:**	Well mixed milking
– **Fat extraction:**	Cream separation and solvent partitioning
– **Gel permeation chromatography:**	Bio Beads SX3; elution volumes 95.0–160.0 ml cyclohexane/ethylacetate 1:1
– **Column chromatography:**	Florisil with 3% H_2O; elution with 3% ethylacetate in light petroleum/dichloromethane 4:1
– **Gas chromatography:**	Fused silica capillary DB5, 30 m x 0.25 mm; film thickness 0.1 µm; splitless injection; temperature programme
– **Mass spectrometry:**	Electron impact ionization at 70 eV; basis ion at m/z = 153.2(phthalic acid anhydride HD_4^+)
– **Limit of detection:**	0.2 µg/kg milk
– **Limit of determination:**	0.6 µg/kg milk
– **Recovery:**	88 %

5_018/97 nb

Figure 2

2.2 Nitro musk compounds

A possible non-specific background contamination in milk fat can arise from detergents in dairying and from the perfumes of the laboratory staff when performing the analytics. The concentrations

observed were in the low ng/kg-fat-range for the 5 compounds under study and were expected not to disturb the follow-up of the excreted amounts after oral supplementation. The molecular formulas of the 5 nitro musk compounds are given in figure 3:

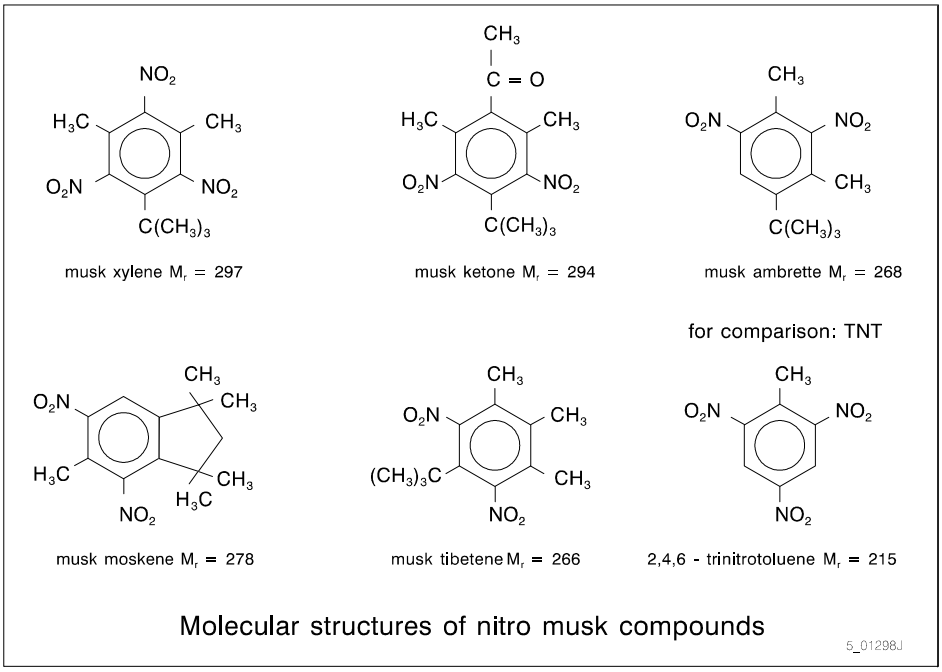


Figure 3

The substances were individually dosed in a daily dose of 150 µg/cow over 15 days. The dose was dissolved in toluene and orally administered via a starch-filled gelatine capsule after the morning milking. The analysis with GC/MS-NCI determination in the final step was carried out according to the following scheme:

Analysis of nitro musk compounds in milk fat	
– **Sampling:**	Cream separation from well mixed milking
– **Fat extraction:**	Solvent partitioning between water, acetone, n-hexane
– **Gel permeation chromatography:**	Bio Beads SX3; elution volumes 115–145 ml cyclohexane / ethylacetate 1:1
– **Column chromatography:**	Silica gel with 1,5% (w/w) water. Elution with toluene/n-hexane 35:65 (v/v) or toluene (musk ketone, -ambrette only)
– **Gas chromatography:**	Fused silica capillary DB5, 30 m x 0.25 mm, splitless injection; temperature programme
– **Mass spectrometry:**	Chemical ionization with negative ion recording, methane as reactant gas
– **Limit of detection:**	0.02 µg/kg milk fat
– **Recovery:**	82–95 %

5_...../98 nb

Figure 4

3 Results and discussion

3.1 Diethylhexylphthalate

Despite the fact that phthalic acid esters show a significant bioaccumulation in the lower links of the aquatic food chain and additionally feature an obvious geoaccumulation the dose was chosen relatively high as the ester-bond C-O-R was expected to be unstable in the rumen of the cows dosed. That this assumption was correct can be seen from the typical excretion curves as given in figure 5.

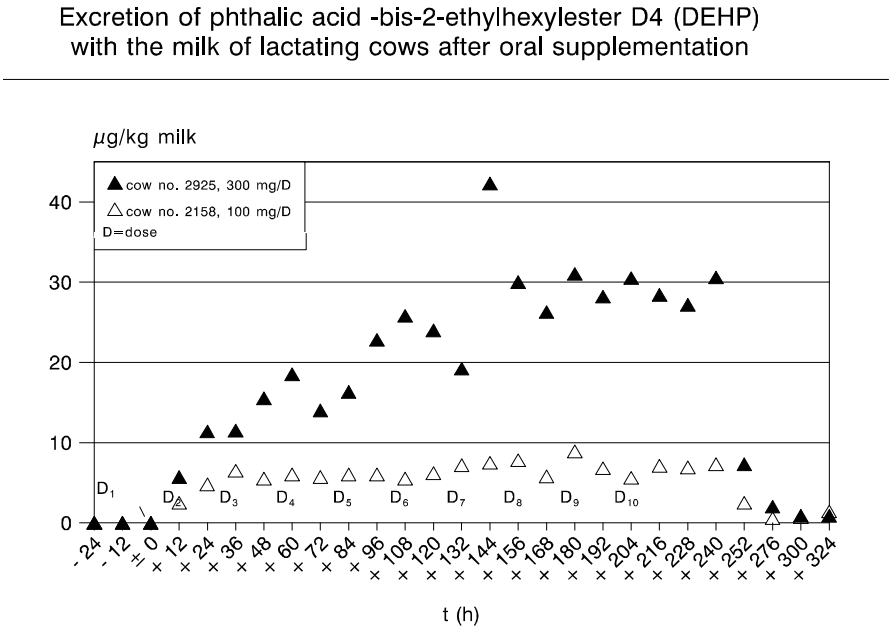


Figure 5

The plot exhibits a dose-related excretion with an equilibrium in the dosing period. The observed peak concentration for the cow receiving 300 mg DEHP-D_4/day results from an erraneously overloaded gelatine capsule and reflects nearly double the dose. As compared to the daily dose of 100,000 to 300,000 µg the excretion in the order of less than 10 to about 30 µg/kg of milk is only low. Blood, taken 22 h after the preceeding dose, exhibits only traces of DEHP-D_4, so that a rapid metabolism beginning in the rumen can be concluded. The carry-over rates as can be derived from dosage and daily milk yield are summarized in figure 6.

As compared to other lipophilic substances the carry-over rate is untypically low.

Carry-over rates of phthalic acid-bis-2-ethylhexylester (DEHP) into milk

Experimental results from feeding assays: **0.08 – 0.19 %** in the daily milking

25.3 kg/d cow, 100 mg DEHP/dose, steady state: **0.18 %**

28.2 kg/d cow, 300 mg DEHP/dose, steady state: **0.19 %**

Rounded up figure for balancing and eco-chemical modelling:

$$\textbf{0.2 \%} \quad \text{or} \quad \frac{\text{daily intake}}{\textbf{500}}$$

For comparison:

Aflatoxin B_1/M_1:	$\frac{1}{50}$
Dioxins (max):	$\frac{1}{2}$
PCBs (max):	$\frac{1}{1}$
Lead, mercury:	$\frac{1}{1000}$

5-023/97 nb

Figure 6

3.2 Nitro musk compounds

The daily dose was chosen to be low due to the only occasional environmental impact on dairying. The resulting dose per kg bodyweight (b.w.) of the cows was in the order of 240 ng. Nevertheless, a lipophilic substance should build up a stable excretion on milk fat base. The observation, however, was contradictory as can be seen for 4 compounds from figure 7.

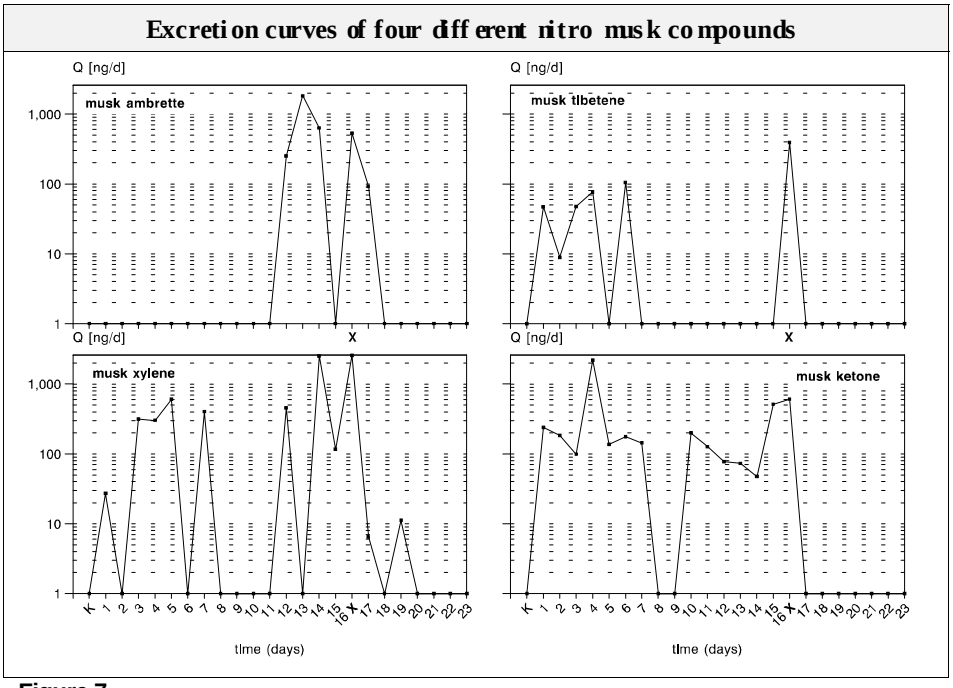


Figure 7

The y-axis shows the excretion with milk fat over the time. The plots exhibit practically only random findings, far away from the formation of a stable plateau. Only musk moskene exhibited a curve like excretion pattern. As the milk fat was analysed in real time, the excretion was continuously observed and the decision made to provocate the excretion with a single provocative dose of 15 mg. This event is marked in the plots with an X. Depending on the type of the nitro musk compound this dose was answered with more or less clear echoes. The integrated carry-over rates over the dosing period are compiled in figure 8.

Carry-over rates for nitro musk compounds into milk (lactating cows)

Compound	Carry-over rates (%)		
	after 1st dose (150 µg)	for 15 x 150 µg/d	after provocative dose 1 x 15 mg (+ 15x150 µg)
Musk ambrette	<0.01	0.019	3.55
Musk tibetene	0.031	0.008	2.61
Musk xylene	0.018	0.042	17.1
Musk ketone	0.160	0.028	4.04
Musk moskene	0.089	0.033	2.33

Figure 8

These absolutely low percentages indicate no transfer risk for occasionally low doses taken up by the dairy cow. The considerably higher rates after the provocative dose are of no environmental significance.

4 Conclusions

4.1 Diethylhexylphthalate

The conclusions that can be derived from the feeding assay support the thesis that the secretory contamination of milk with DEHP is low and stands clearly in the background of other post-secretory contaminatory possibilities. A balancing modelling on the basis of a uniform DEHP deposition after the environmental release of the production aliquot typical for Germany features the results given in the following figure 9.

As the "normal" DEHP content is in the order between 5 and 50 µg/kg of milk, the other sources must be abundant. Another modelling with the assumption of a 10-µg/kg-gain from the milking equipment seems to be realistic as compared with the observed brittleness of the rubber parts after their service period (fig. 10).

The summarized conclusion either from the secretory or post-secretory contamination of milk with DEHP is given in figure 11.

Ecological-chemical balancing of DEHP* transfer into milk	
Annually atmogenic deposited quantity:	~ 500(+) µg/m²
	or ~ 1.4 µg/m² • d (e.g. for Germany >180mt/year)
Grazing area / cow / day:	18.75 m²
Theoretically deposited DEHP qty:	18.75 x 1.4 = 25.70 µg/day
Carry-over rate:	0.2 %
DEHP in the daily milking:	0.05 µg
or	
DEHP per litre of milk:	3.2 ng (in a 16 kg milking/day)
Conclusion: The **secretory contamination** of milk is **negligibly low** from the ubiquitous background contamination	
* Phthalic acid-bis-2-ethylhexylester	5_024/97 nb

Figure 9

Approximative balancing of the postsecretory milk contamination with DEHP* during milking		
Assumption:	**5 % DEHP** in the rubber parts of cluster and milk tubes (**industry secret**)	
Weight of milk-contacted rubber parts:	1900	g
Quantity of DEHP (5 %):	95	g
Milk flow through cluster and tubes/day (10 cows, 20 kg milk each):	200	kg
Additional DEHP contamination through machine milking (estimate):	10	µg/kg
Quantity of DEHP in the daily milking:	2000	µg
Percentage of DEHP from rubber parts/day:	**0.0021**	**%** (daily wasting)
Wasting of DEHP during one lactation period per milking machine (300 days):	0.63	%
The wastings of DEHP during milking **seem plausible by their order of magnitude**. The figure is supported by the observed hardening and brittleness.		
* Phthalic acid-bis-2-ethylhexylester		5_027/97 nb

Figure 10

Conclusions to the carry-over of DEHP* into the milk
– The carry-over rate is rather low at 0.2 %
– An abundant secretory contamination of milk is unlikely to occur from the low carry-over rate
– Despite fat solubility of the substance no storage in the body of the cow is observed
– The concentrations in plasma reach at maximum 0.07 % of the dosage
– The half-life time in the milkings is less than 24 hrs
– The observed DEHP concentrations in liquid milk between 10 and 50 µg/kg result from postsecretoric contamination
– Before the background of an ADI figure between 0.6 and 1.0 mg/kg body weight a transmissible risk is unlikely for man
* Phthalic acid-bis-2-ethylhexylester 5_025/97 nb

Figure 11

4.2 Nitro musk compounds

The statement seems to be clear that nitro musk compounds show an untypical behaviour in the ruminant, even after a relatively high dosage. Still there are open questions regarding the fate of the amounts applied. The conclusion that the nitro musk compounds are degraded to a significant extent in the rumen can be supported with the only parenteral application of a phenolic nitro fasciolicide to become therapeutically effective. The last figure summarizes the results so far (figure 12).

Conclusions to the carry-over of nitro musk compounds into the milk
– The carry-over rate is substance-dependent and after low dosage (~ 240 ng/kg b.w.) between 0.008 and 0.04 %
– A provocative dose of 24 μg/kg b.w. is answered with carry-over rates between 2 and 17 %.
– Nitro musk compounds show no persistence in milk fat
– Before main exposition of humans occurs through direct dermal absorption from cosmetics and to a far lesser content from fresh water organisms

Figure 12

5 Summary

Phthalic acid esters, especially diethylhexylphthalate (DEHP) and nitro musk compounds are synthetic environmental chemicals which can become into contact with dairying and are in the case of DEHP regularly found in milk and milk products. Both groups are found in human adipose tissue as a result of foodborne and cutaneous exposition. To quantify the secretory carry-over into milk (fat), both DEHP-D_4 and five nitro musk compounds (musk ambrette, – tibetene, – xylene, – ketone, – moskene) were orally supplemented to lactating cows and the excretion with milk quantified. The carry-over rate for DEHP is little less than 0.2 %, the observed ratios for nitro musk compounds are between 0.08 and 0.04 % of the dose administered. In the case of DEHP the findings indicate the predominant role of post-secretory milk contamination. Both types of compounds build up no depots in the dairy animal.

Acknowledgement

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Literature

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