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Initial Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Feed (materials)

Addendum to Report: 03/0027745

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APPENDIX

SUMMARY AND CONCLUSIONS

Feed materials like dried grass, may contain elevated levels of Poly Aromatic Hydrocarbons (PAHs). These compounds may end up in edible products and as such impose a possible risk for consumers. In 2003 a carry-over study on Poly Aromatic Hydrocarbons (PAHs) from feed to milk was initiated, the results were presented in the internal report ASG 03/0027745 by Kan et al., 2003. The current report is an addendum to the study report.
In this addendum, an initial risk assessment on Poly Aromatic Hydrocarbons (PAHs) for consumers is presented. For this, a toxicological profile of PAHs has been made, and data from the report of Kan et al (2003) and the Control program animal feed have been used. The main issue that is addressed in this addendum is the availability and health-related effects of PAHs originating from animal feed for the consumer.
Although very little data are available on carry-over rates, it is generally assumed that PAHs are not transferred from animal feed to animal products such as milk. In this report, it is shown that contrary to this assumption, certain PAHs are transferred as native compounds to milk, in particular those PAHs with less than five rings. In addition, it appears that metabolites of some PAHs are transferred to milk. The carry-over of metabolites and its toxicological relevance are generally underestimated in risk assessments. The carcinogenic properties of PAHs can be contributed to the PAH metabolites, however a complete toxicological profile of the metabolites is not available. The metabolites of PAH have not been considered in monitoring programs yet, thus only limited data are available, which is severely hampering the risk assessment.
Since several non-carcinogenic PAHs (of lower molecular weight) are transferred intact to milk, it is interesting to focus also on the non-carcinogenic properties of these PAHs. In this document, an initial (worst–case) risk assessment using several scenarios has been performed to illustrate this. The results indicate that the intake of PAHs by cattle can be in the mg-range. Because of the low transfer rates, the calculated intake by humans from milk is at least a factor 1000 under the chronic Reference Dose (RfD) for non-carcinogenic effects. Interestingly, calculated transfer of high-molecular PAHs results in exceeding the 'virtually safe dose' for Benzo(a)Pyrene in several worst case scenarios.

Conclusions:

Toxicology

- Data on non-carcinogenic effects of Poly Aromatic Hydrocarbons (PAHs) are very limited. Similar applies to the oral carcinogenic potential of PAHs other than Benzo(a)Pyrene (BaP).

Scenarios

- The experimental scenario ASG seems representative of a worst case scenario;
- Of the PAHs present in grass pellets, fluoranthene contributes the most to the calculated total PAH concentration in milk;
- Of the PAHs present in fresh grass, pyrene contributes the most to the calculated total PAHs concentration in milk.

Transfer from feed to animal products

- Data on transfer rates of PAHs to cow's milk and other animal products are very limited; Metabolised PAHs are generally not considered in the transfer of PAHs from feed to food. It is likely that PAHs with more than 5 rings are transferred as metabolites;

- Artificially dried roughage feed, such as grass pellets, contribute the most to the total concentration of PAHs in milk.

Human oral exposure

- The calculated human intake of PAHs from animal feed via milk is far below the RfDs set for non-carcinogenic effects;
- Calculated transfer of high-molecular PAHs from feed to cows milk results exceeding the 'virtually safe dose' for BaP up to a factor 2.75 for humans consuming this milk. Based on the data in literature, this could be the result from transfer of BaP metabolites.

Animal oral exposure

- Calculated intake of PAHs by cows is 65 to 1000 times higher than the calculated intake by humans, which could indicate a potential risk for animal health.

Control program

- The current analysis of PAHs in food should not be expressed solely in BaP equivalents;
- Analysis of PAHs in animal products should also include metabolites of PAHs;
- For use of the monitoring results in risk assessment, the design of the control program should be adapted.

1 INTRODUCTION

The level of contamination of the Dutch animal feed is monitored by means of the Control program animal feed (following Regulation 882/2004). Up until 2005 not only undesirable substances as defined by 2002/32/EC and amendments were included but also contaminants without a legal commodity limit for animal feed. Inclusion of these type of contaminants into the Control program in future years should be based on risk-assessment. Four groups of contaminants were identified for which a risk-assessment would be needed: e.g. nickel (in mineral mixes), extraction compounds, mineral oils and Polycyclic Aromatic Hydrocarbons.
The scope of this project was to perform an initial risk assessment in order to be able to decide on the inclusion of PAHs in future control programs and give recommendations for further research and policy making.
In 2003 it was recognized that data on the carry-over of PAHs from animal feed to milk were very limited. In order to provide new data an animal study was initiated to study the carry-over from animal feed to milk. Cows were fed diets with a level of contamination that was thought to be representative of the situation in practices. However, an evaluation of the results from the Control program was not performed.
The current report is an extension of the above mentioned study. An initial risk-assessment of the PAHs (shown in table 1) is reported including an exposure assessment of food producing animals. In addition, the contribution of the consumption of animal products to the PAH burden of consumers are briefly mentioned.

Name	Classification	# Rings	CAS nr	BaPEF
Acenaphthylene	Low molecular	2	208-96-8	0.01
Acenaphthene	Low molecular	2	83-32-9	0.001
Fenanthrene	Low molecular	3	85-01-8	0.001
Fluoranthene	Low molecular	3/4	206-44-0	0.01
Pyrene	High molecular	4	129-00-0	0.001
Benzo[a]anthracene	High molecular	4	56-55-3	0.1
Chrysene	High molecular	4	218-01-9	0.01
Benzo[b]fluoranthene	High molecular	4/5	205-99-2	0.1
Benzo[k]fluoranthene	High molecular	4/5	207-08-9	0.1
Benzo[a]pyrene	High molecular	5	50-32-8	1
Indeno[1,2,3-c,d]pyrene	High molecular	5/6	193-39-5	0.1
Dibenzo[a,h]anthracene	High molecular	5	53-70-3	1

Table 1. Details of PAHs evaluated in this document. BaPEF = factors expressing relative carcinogenic potency as compared to Benzo(a)Pyrene (taken from Hoogenboom et al., 2003). See also paragraph 3.

1.1 Definition

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused aromatic rings made up of carbon and hydrogen. At ambient room temperatures PAHs are solids. Generally, they have high melting and boiling points, low vapour pressure, and very low water solubility. PAHs are very lipophilic, and chemically rather inert. They are used as intermediates in the production of plastics and plasticizers, pigments and dyes, and pesticides. The largest emissions of PAHs into the

environment result from incomplete combustion of organic materials during industrial processes and other human activities.
Many of the PAHs are considered genotoxic carcinogens, depending on their molecular structure. PAHs are usually distinguished in low molecular weight PAHs (2 or 3 aromatic hydrocarbon rings) and high molecular weight PAHs (4 aromatic hydrocarbon rings and more), from which the latter are less acutely toxic but more carcinogenic and teratogenic (Baars et al., 2001). Some organisations (like US-Environmental Protection Agency EPA) consider PAHs with 4 rings also to be low-molecular, in this document however, the first definition will be used.
For the general population, the major routes of exposure to PAHs are from food and ambient and indoor air. Food can be contaminated by environmental PAHs that are present in air (by deposition), soil (by transfer) or water (by deposition and transfer), or during processing and cooking.
Processing of food (such as drying and smoking) and cooking of foods at high temperatures (grilling, roasting, frying) are major sources of PAH contamination (Guillén *et al.*, 1997; Phillips, 1999). Cigarette smoking increases PAH exposure significantly (World Health Organisation WHO, 2002).

1.2 Occurrence

Kan et al. (2003) reviewed the occurrence of PAHs in animal products. In France, PAHs have been found in milk at total levels of 37 and 27 ng/g fat (Grova et al. 2000, 2001). Concentrations were not significantly different between milk from cows in a highly industrial area and a relatively 'clean' rural area. In another study, concentrations up to 4 µg/kg and 125 µg/kg were found in respectively locally produced and imported cheese in Finland. Concentrations up to 70 µg/kg were found in meat. In uncooked foods, the average background values are usually in the range of 0.01-1 µg/kg (Scientific Committee on Food SCF, 2002).

2 TOXICOLOGICAL PROFILE

Evidence that mixtures of PAHs are carcinogenic to humans is primarily derived from occupational studies of workers following inhalation and dermal exposure. No data are available for humans following the oral (food) route of exposure. There are few data from animal studies on the oral toxicity of PAHs other than Benzo(a)Pyrene (BaP), and most studies focus on the carcinogenic properties. For BaP there are two oral carcinogenicity studies, one in mice and one in rats. In available studies animals were mostly exposed to contaminated drinking-water. The acute oral toxicity of PAHs ranges from very to moderately toxic (50 to 1000s mg/kg bw) in rats.
When applied on the skin, many PAHs are cancer-causing, producing tumours in epithelial tissues in "practically all animal species tested" (Agency for Toxic Substances and Disease Registry ATSDR, 1995). Carcinogenic PAHs in general show clear positive effects in genotoxicity tests and from DNA-adducts in exposed animals.
Many PAHs, and in particular their hydroxy metabolites, may induce (anti)estrogenic effects. Gozgit et al. (2004) found that a metabolite of BaP was capable of inducing estrogenic response genes *in vitro* in human breast cancer cells. In addition, Van de Wiele et al. (2005) discovered that colon microbiota transformated PAHs to metabolites, which induced a estrogenic response in a yeast estrogen bioassay. For their study, they used a gastro-intestinal simulator. Whether the observed effect occurs in vivo, when several enzymes could interfere with the transformation, is yet unclear.
The immunotoxicity of PAH has been known for a number of years. The immunotoxic effect most often reported following exposure to PAH is immunosuppression. It should be noted that most studies on the immunotoxicity of PAH have used parenteral administration and that most of the available data consider only a few selected substances, benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene being most widely used (SCF, 2002).
PAHs bind to the Aryl-hydrocarbon (Ah) receptor, and could thus be capable of inducing dioxin-like effects. There are indications that this mechanism plays an important role in the carcinogenicity of PAHs. Other effects in terrestrial organisms include adverse effects on reproduction and development (SCF, 2002, ATSDR, 1995).

Benzo(a)Pyrene (BaP) is the most potent compound of the PAHs based on carcinogenicity. Relative potencies of carcinogenic PAHs to BaP have been determined by comparison of data which come primarily from dermal studies. The order of potencies is consistent, and this scheme therefore can provide an indicator of PAH potency relative to BaP, expressed as BaPEQ (BaP equivalents). Controversy exists concerning the use of this expression of relative carcinogenicity, since not all PAHs induce cancer via identical mechanisms. Also, data on oral studies are scarce and absorption and metabolism may play an important role in the effects. SCF has decided that benzo(a)pyrene is a good indicator of the PAH content. Based on studies with coal tar, they conclude that multiplication of the BaP-content by a factor of 10 will give a good indicaton of the total potency of the mixture. However, this refers only to the high-molecular PAHs (SCF 2002). For reasons discussed in paragraph 5, the results in this initial risk assessment will be evaluated in weight only*.
Please note that this assessment only deals with oral exposure of consumers via the route of milk and dairy products.

* Recently the discussion on expression of concentrations of PAHs was continued during a meeting of an expert group organised by JRC and EFSA. In addition EFSA announced to develop a database that should contain PAH levels in food commodities (http://www.efsa.eu.int/science/data_collection/pah/1168_en.html)

2.1 Combination toxicology

In carcinogenic mechanisms, PAH mixtures have been shown to induce differential effects in covalent DNA binding when applied to mouse skin in different combinations. Effects varied from synergism to antagonism, depending on the applied combination (Hughes and Philips, 1990). Like dioxins, PAHs can have combination effects like synergism or antagonism when binding to the ArylHydrocarbon receptor and induce dioxin-like effects (Chaloupka et al., 1993). After oral exposure, BaP has shown to enhance the immune response to the food allergen ovalbumine in mice (Kadkhoda et al., 2004).

2.2 Absorption, Distribution, Metabolism and Excretion (ADME):

Absorption of PAHs from the gastro-intestinal tract appears to vary per animal species. BaP absorption reached 89-99% after oral (food) exposure of rats (Rabache et al., cited by Kan et al, 2003). Another study in rats showed that first a direct absorption occurs 1-2 hours after feeding. After 3-4- hours a second increase in serum concentration occurs due to entero-hepatic circulation (Van Schooten et al. 1997, cited by Kan, 2005). In contrast, a study from Grova et al. (2002) showed that activity from radio-labeled BaP was not traced in blood and milk from orally exposed lactating goats. Hoogenboom et al. (2005) concluded from this study that the heavier PAHs are apparently not absorbed from the gastro-intestinal tract (and transferred to milk). Since the rat seems more relevant as a model for human uptake, it is considered that in humans PAHs may be readily absorbed from the gastro-intestinal tract.

The rate of distribution of PAHs can be influenced by the presence of other (fatty) compounds that may enter the body at the same time with PAHs. PAHs can enter all the tissues of the body that contain fat. They tend to be stored mostly in the kidneys, liver, and fat. Smaller amounts are stored in the spleen, adrenal glands, and ovaries (ATSDR, 1995).

Detoxification of PAHs is complicated, and is performed by various enzymatic and nonenzymatic reactions. PAHs are converted to arene oxide intermediates followed by formation of derivatives of *trans*-dihydrodiols, phenols, and quinones. These intermediate products are known to be toxic, carcinogenic, and/or mutagenic. Results from animal studies show that PAHs do not tend to be stored in the body for a long time. Most PAHs that enter the body leave within a few days, primarily via feces and urine (ATSDR, 1995).

2.3 Toxicological reference limits

Based on existing NOAELs for non-carcinogenic effects of PAHs, the US EPA (Integrated Risk Information System IRIS, 2002) has derived Reference Doses (RfDs) for chronic oral exposure for acenaphthene, anthracene, fluoranthene, fluorene, naphthalene and pyrene. Large safety factors were used by the US EPA because of the limited databases available and the use of subchronic studies and not chronic studies for the derivation. According to EPA it is the carcinogenic and genotoxic potential of PAH that is critical for the risk assessment, because exposure to PAH in food is almost exclusively to a mixture of PAH which includes genotoxic and carcinogenic PAH. The non-carcinogenic effects of individual components are generally considered not to be relevant for the assessment of the risk of such mixtures. However, there are more and more data that indicate that in the case of milk production, only the low-molecular (non-carcinogenic) PAHs are transferred from feed to the milk in their original form (Lutz et al., 2005, Grova et al., 2002). Therefore, a risk assessment using TDIs based on non-carcinogenic effects could be useful. An orientation on this matter using data from the Control program animal feed has been performed and is described in paragraph 6.

Based on carcinogenic potential, the following organisations have derived 'reference' limits:

World Health Organisation (WHO, 1998, 2003)

The 1993 Guidelines (retained in 1998) concluded that there were insufficient data available to derive drinking-water guidelines for PAHs other than BaP. The study used for deriving a guideline value was that in which CFW mice were fed BaP in the diet and an increase of stomach tumours associated with an increase in the ingested concentration of BaP was observed. The tumour incidence data have been extrapolated using the two-stage birth - death mutation model. The estimate of the upper bound on the low-dose risk was 0.46 (mg/kg of body weight per day). The derived guideline value for BaP, corresponding to an upper-bound excess lifetime cancer risk of 10^{-6}, was calculated to be 0.07 µg/litre. Although a health-based value for fluoranthene was calculated in the addendum, it was significantly above the concentrations found in drinking-water, and it was concluded that, under usual conditions, the presence of fluoranthene in drinking-water does not represent a hazard to human health; thus, the establishment of a guideline value for fluoranthene was not deemed necessary.

Agency for Toxic Substances and Disease Registry (ATSDR)

ATSDR did not derive chronic oral minimal risk levels (MRLs) for polycyclic aromatic hydrocarbons (PAHs) because there are no adequate human or animal dose-response data available that identify threshold levels for appropriate non-cancer health effects.

US - Environmental Protection Agency (EPA)

Using the oral slope factor of 7.3 per mg benzo[*a*]pyrene/kg bw/day for the carcinogenic risk from benzo[*a*]pyrene exposure as developed by US EPA (IRIS, 2002) an oral "virtually safe dose" (VSD) for benzo[*a*]pyrene of 0.14 ng /kg bw/day was calculated for a cancer risk level of $1x10^{-6}$ via linear extrapolation.

European Food Safety Authority (EFSA) - Scientific Committee on Food (SCF)

The SCF have concluded in 2002 that since a number of PAH have been demonstrated to be genotoxic and carcinogenic, the existence of a threshold could not be assumed and therefore, the Committee did not establish a safe exposure limit. It was recommended that exposures to PAH should be as low as reasonably achievable. EFSA has scheduled a review of PAH data for April 1st 2007.

Rijksinstituut voor Volksgezondheid en Milieu (RIVM)

Based on a 3-year oral study in rats, RIVM derived a limit of 5 ng BaP/kg bw/day (from a LOEL of 10 mg/kg bw/day for tumour incidence) which would correspond with a cancer risk level of $1x10^{-6}$ (Kroese et al., 2001). On the basis of the available data on occurrence and carcinogenic potency of PAHs in the Dutch diet, the authors suggested to apply a correction-factor of 10 for conversion to a VSD for all dietary PAHs, resulting in a VSD of 0.5 ng B[a]P/kg bodyweight per day, taking B[a]P as PAH indicator.

Based on non-carcinogenic potential, the following organisations have derived reference limits:

US-EPA

For acenaphtene, US-EPA (1994) derived a RfD of 0.06 µg/kg bw/d based on a subchronic NOEL of 175 mg/kg bw/d for hepatotoxicity in mice.
For fluoranthene, US-EPA (1993) derived a RfD of 0.04 µg/kg bw/d on a subchronic NOEL of 125 mg/kg bw/d for nephropathy, increased liver weights, hematological alterations, and clinical effects in mice.
For pyrene, US-EPA (1993) derived a RfD of 0.03 µg/kg bw/d based on a subchronic NOEL of 75 mg/kg bw/d for Kidney effects (renal tubular pathology, decreased kidney weights) in mice.
No RfDs were derived for other PAHs relevant to this document.

RIVM

RIVM (2001) derived a chronic TDI of 40 µg/kg bw/d for phenanthrene. Although they considered this compound to be carcinogenic, its carcinogenic potency was considered to be extremely low and therefore the TDI for non-carcinogenic PAHs was applied. This TDI was derived from the evaluation of Total Petroleum Hydrocarbons, where an overall TDI of 40 µg/kg bw/d was set for non-carcinogenic aromatic compounds with equivalent carbon index (EC, this index is equivalent to the retention time of the compounds on a boiling point GC column (non-polar capillary column), normalized to the n-alkanes) of >9 to 16, and 30 µg/kg bw/d for those with equivalent carbon numbers of >16 to 35 (see document on Total Petroleum Hydrocarbons for details).

3 HUMAN ORAL EXPOSURE

In a study of De Vos et al. (1990, cited by SCF 2002) a worst case scenario was used for calculation of oral PAH intake, resulting in a daily exposure of 420 ng/kg bw BaP for Dutch consumers. In this study, an average intake of 4.8 ng/kg bw/day BaP and 284 ng/kg bw/day total PAHs was calculated (cited by COT, 2002). The major contributors to the daily BaP intake were oils and fats (47%), cereals (36%) followed by sugar and sweets (14%). These results were in line with other studies of the UK diet (Dennis et al., 1983, cited by SCF 2002). The relatively high contribution of oils and fats was, at least partly, attributed to the well-known elevated PAH concentrations present in vegetable oils. However, measurements for the study of De Vos et al. were performed from 1982-1986, thus 20 years ago. The more recent total diet study in the UK (COT, 2002) showed that the contribution from oils and fats to the total PAHs intake was far less than in previous studies. However, the contribution from milk and dairy products had increased to 12% and 9% respectively. In addition, partitioning of PAHs in the total PAH uptake was changed. Although the composition of the diet samples were different from the earlier studies, the increase in milk contribution is relevant to the risk assessment performed here. The contribution from other animal products is far less, and will therefore not be included in the initial risk assessment. The focus of this document will be on human oral exposure via milk and dairy products.

4 MEASUREMENTS IN FEED (MATERIALS)

To evaluate the effect of the method of expression of PAH concentrations on the interpretation of results, data on PAHs in dried grass were evaluated based both on benzo(a)pyrene equivalents (BaPEQs) and based on weight. Analysis in the Netherlands of artificially dried grass by RIKILT showed a range from 0.57-18.87 ng BaPEQ/gr dried product in 2000 and 2001. In 2002/2003 this range varied from 0-7.3 ng BaPEQ/g in dried grass and 0.8-180 ng BaPEQ/g in grass pellets (data not shown). As could be expected, the contribution of high-molecular PAHs to the total BaPEQs was much higher than the low-molecular PAHs, as the BaPEQs are based on carcinogenic properties. In weight, the contribution of low-molecular and high-molecular PAHs is equal on average, in dried grass the contribution from low-molecular PAHs seems to be slightly higher (average 58.3 and 41.7%).
The previous indicates that expression of PAH concentration in BaPEQs may result in a underestimation of the total concentration of PAHs. To obtain a clear image of the distribution of PAHs in feed ingredients measured for the Control program animal feed, the data will thus be expressed in weight only.

Data from the Control program animal feed have been processed and results are shown in table 2. Matrices were categorised and maximum and median concentrations per category were determined. Details on this calculation can be found in Appendix A.

Category		ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR
1	Flakes	34.78	2.00	3.17	2.00	1.74	0.38	4.79	0.14	76.16	15.83	1.10	31.87
2	Oils/fats	9.20	6.50	7.60	9.80	9.50	7.20	9.00	6.30	9.00	9.60	9.10	9.50
3	Citrus	5.30	2.50	7.60	9.60	6.50	2.40	9.20	0.10	0.10	0.10	6.90	0.10
4	Roughage (dried)	13.36	12.94	14.30	9.80	10.18	8.80	15.07	8.40	80.80	157.40	8.50	98.20
4a	Roughage (silage)	1.20	1.80	8.20	7.90	9.10	4.30	0.72	1.20	3.90	2.70	5.40	1.90
5	Other	1.10	0.66	6.00	7.30	6.00	3.10	5.30	0.88	5.10	4.60	5.00	2.90

Table 2. Maximum concentration in feed ingredient categories as measured in the Control program animal feed from 2000-2004. Concentrations in µg/kg. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

4.1 Commodity limits

There are only few limits on PAHs in feed and feed ingredients. As a result of the mixing of diesel oil with palm oil, the Product Board Animal Feed set a limit of 50 µg BaPEQ/kg fat for vegetable oil and oil derived waste products. In addition an action limit of 15 µg BaPEQ/kg fat was established (PDV, 2001).

5 TRANSFER FROM FEED (MATERIALS) FOR ANIMALS TO PRODUCTS FOR HUMAN CONSUMPTION

Based on the often high levels in dried grass, Kan et al. (2003) performed an exploring study on the transfer of PAHs from feed to milk in lactating cows. They reported very low transfer of PAHs to milk. Acenaphtene, phenanthrene, fluoranthene, pyrene and chrysene were detected to some extent in the milk, but the more heavy PAHs were not present at levels above the detection limit of 0.1 ng/g fat. Analysis did not include metabolites. Grova et al. (2002) detected traces of low-molecular fluorene-, phenantrene- and pyrene related radioactivity in milk after oral exposure of goats to several radiolabeled PAHs. BaP-related activity was hardly detected in milk (0.2%) and mainly excreted in the feces (88%). This does not exclude entero-hepatic cycling and thus a much larger initial absorption of BaP. A recent study from Cavret and Feidt (2005, 2005a) indicated the importance of metabolism in the fate of PAHs and transfer to milk. In another study, Lutz et al. (2005) did not detect the parent compounds, but found the hydroxy-metabolites from fluorene, phenantrene and pyrene in the milk from cows which were chronically exposed to PAHs through oral soil intake.

Metabolites are usually not included in the 'classical' monitoring schemes of PAHs. It can be concluded from recent studies that it is likely that low(er)-molecular PAHs with less than 5 rings are transferred to milk as native compound after oral exposure. The study of Kan et al. (2003) confirms this assumption. In addition, evidence from literature suggests that even more PAHs are transferred as metabolites (Lutz et al., 2005), possibly including those of the high-molecular PAHs.

6 INITIAL RISK ASSESSMENT

As seen in previous paragraphs, PAHs can transfer from contaminated feed to milk, as native compounds or as metabolites. By consuming this milk, humans can be exposed to the PAHs originating from the feed. To evaluate the possible risk of exposure to these PAHs, an initial risk assessment was performed. For this, various worst-case exposure calculations were performed to estimate the human exposure to PAH via the consumption of milk. Available data from the Control program on PAH concentrations in animal feed was used. On some matrices no or very limited data was available from the Control program, for instance for fresh grass. On this last matrix, a brief literature review was undertaken resulting in data from a few studies.

6.1 Description of scenario's

Using the maximum and median concentrations measured in the defined categories of feed ingredients, concentrations in compound feed were calculated. PAH concentrations in milk resulting from feed intake by cows were calculated using transfer rates derived from the study of Kan et al. (2003) and from the studies of Lutz et al., 2005 and Grova et al., 2002. To calculate possible concentrations in cow's milk, the procedures as introduced by Van Raamsdonk et al. (2004) were used. Human intake was then calculated and compared to the known reference values using standard methods of the European Medicines Agency (EMEA, 1995), RIVM and RIKILT (RIVM/RIKILT Frontoffice, 2005). For details on the method of calculation, see appendix A.

The winter and summer scenarios were based on the availability of feed during the year. In winter, no fresh grass is available, so only silage and dried pellets are used as roughage feed. In summer, the main component in roughage is fresh grass. For comparison with the experimental data obtained by Kan et al. 2003, the feeding regime used in this study was also used as scenario. Feeding regimes in the scenarios are described in the next paragraphs. Details on amounts of feed can be found in table A6 in appendix A.

6.1.1 Scenario Winter

The winter feeding regime as used by Van Raamsdonk et al. 2006 (in preparation) was used. Three winter scenarios were designed because of the relative high levels of PAHs in dried grass pellets. It is assumed that the PAHs are introduced by the drying process, although this remains to be confirmed. Theoretically, farmers in the vicinity of drying facilities will use more dried materials than farmers further away, because of the costs of transportation. In three scenario's, the percentage of silage in the winter feeding regime is replaced in varying proportions by grass pellets to simulate this variety in feed sources. The consequence of this varying use is calculated accordingly. Concentrations in corn silage were assumed to be equal to grass silage (note: only two samples of grass silage were present in database).

Winter 1 (W1): Normal percentages of concentrate were used. Roughage consisted completely of grass and corn silage.

Winter 2 (W2): Normal percentages of concentrate were used. Roughage consisted half of grass and corn silage and half of grass pellets.

Winter 3 (W3): Normal percentages of concentrate were used. Roughage consisted completely of grass pellets.

6.1.2 Scenario Summer

The summer feeding regime as used by Van Raamsdonk et al. (2006, in preparation) was used. Two summer scenarios were designed because of the lack of data from fresh grass samples in the Control program. The scenarios differ in the use of PAH concentrations in fresh grass, for one scenario no data are used (current situation), and for one scenario data is taken from literature. This way the consequence of the current monitoring strategy could be evaluated.

Summer 1 (S1): Normal percentages for concentrate and silage were used. No grass pellets were included in the regime. PAH content of fresh grass was considered to be 0 mg/kg.

Summer 2 (S2): Normal percentages for concentrate and silage were used. No grass pellets were included in the regime. PAH concentrations in fresh grass were taken from Crépineau-Ducoulombier et al. (2004, control site 2), where levels lower than detection limit were assumed to be at the level of detection.

6.1.3 Scenario study ASG

The feeding regime as used in the study of Kan et al. (2003) was used with the data from the Control program. This regime consisted of concentrated feed, silage, and grass pellets.

6.2 Results

Data on intake and PAH levels were used to calculate the exposure of the cows and subsequently the expected levels in milk, based on carry-over rates. These levels were than used to estimate the exposure of the consumer, based on a milk consumption of 1.5 l per day. Calculated human total intake of PAHs for all scenarios is presented in figure 1. From the worst case scenarios based on maximum concentrations in feed ingredients, winter scenario 3 resulted in the highest calculated intake for humans of PAHs. Scenario ASG resulted in the second highest human intake of PAHs.
Based on median concentrations, the scenarios did not result in large differences of PAH intake as compared to the worst case scenarios. Again, winter scenario 3 resulted in the highest intake of PAHs, but by a very slight difference (0.016 µg/kg bw/day for WS3 versus 0.015 µg/kg bw/day for WS1 and WS2). For summer scenario 2, no calculation could be made based on median concentrations, since data were obtained from literature and no range was available.
Distribution of PAHs for the worst case scenarios is presented in figure 2. The calculated intake in the scenarios consisted predominantly of PAHs phenanthrene, fluoranthene and pyrene.

6.2.1 Scenario Winter

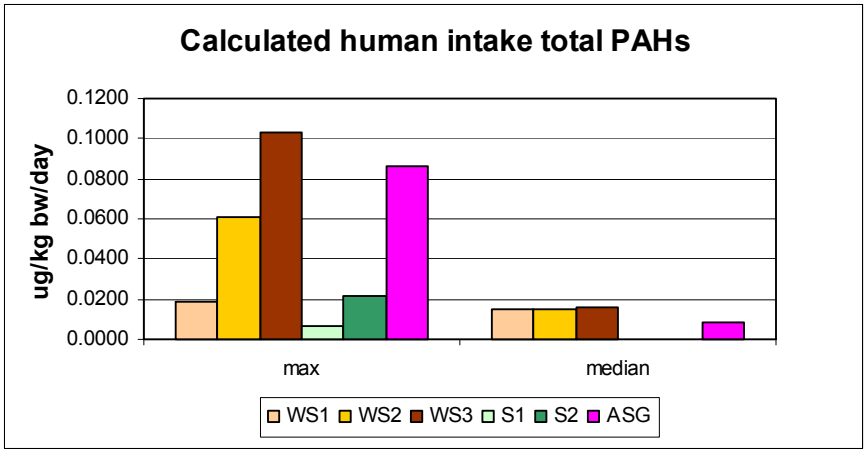
With increasing intake of grass pellets in winter scenarios 1-3, the contribution of phenanthrene decreased, while the contribution of fluoranthene and pyrene increased. Distribution of the higher molecular PAHs indicated an increase in the contribution of chrysene and dibenzo(a)anthracene with increasing intake of grass pellets.

6.2.2 Scenario Summer

Summer scenarios 1 and 2 show the impact on risk assessment of calculation with or without data for fresh grass. Using data from literature, the contribution of fresh grass to the total intake of PAHs is 73%. In the summer scenarios, total intake also mainly exists of phenanthrene, fluoranthene and pyrene.

6.2.3 Scenario study ASG

The scenario study ASG results in the second highest calculated total intake of PAHs. Distribution of PAHs is similar to the winterscenario 3.



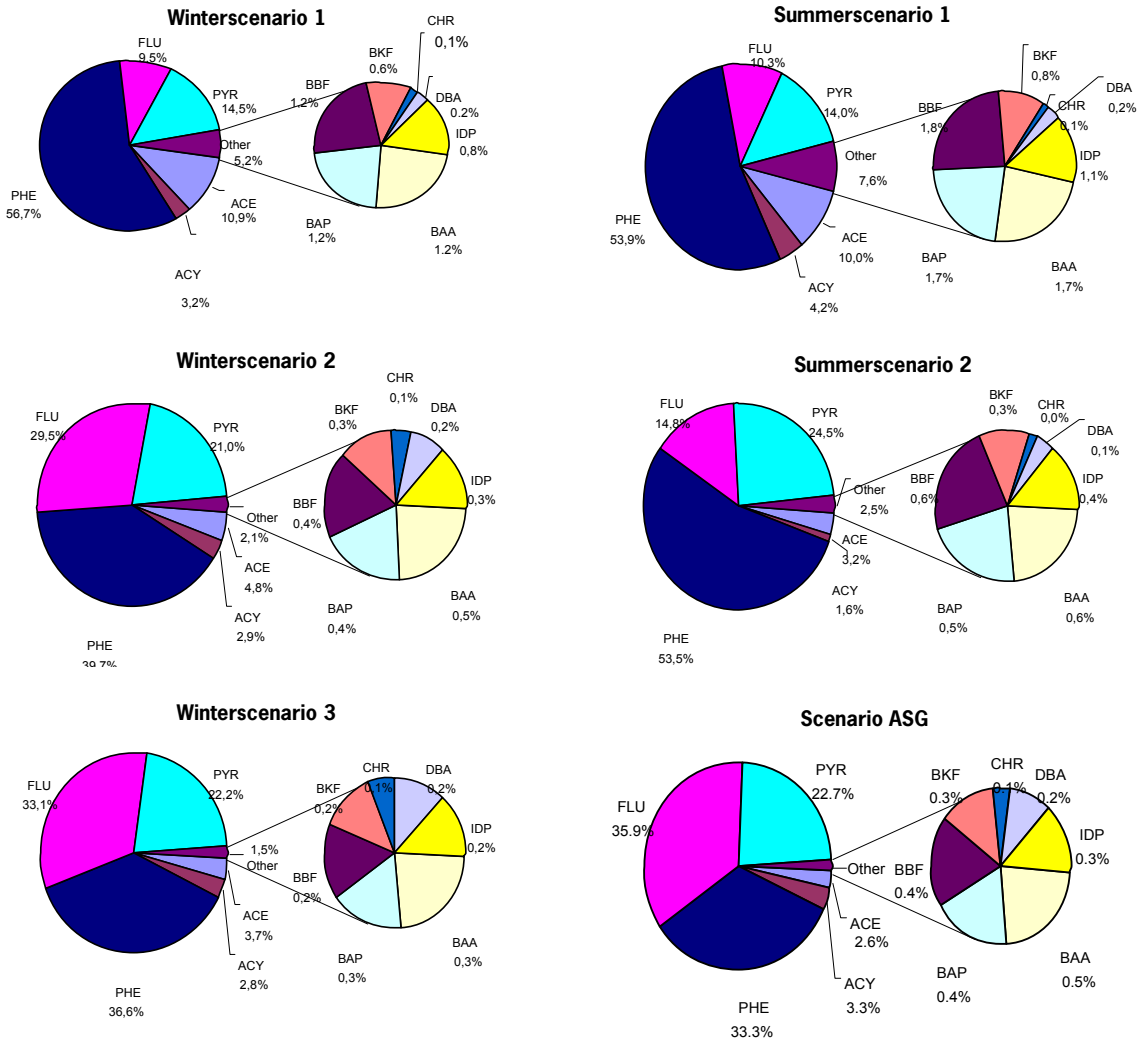


Figure 2. Contribution of individual PAHs to the calculated concentrations in milk for the worst case scenarios*. Distribution in percentages. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.*

6.2.4 Comparison of calculated PAH intake with reference values

Although the lower molecular PAHs contribute most to the total calculated human intake, the RfDs for these PAHs are not exceeded according to this calculation (Table 3), the margin is over a factor 1000. In contrast, the lowest 'virtually safe dose' (0,14 ng/kg bw/day, US-EPA) for benzo(a)pyrene is exceeded by the calculated human intake by a factor of up to 2.75 for scenario ASG (figure 3). Based on medians, this 'safe' dose is exceeded for winterscenario 1 and scenario ASG. The calculated intakes do not exceed the VSD of 0,5 ng/kg bw/day as set by RIVM, either based on maximum or median concentrations.
The low molecular PAHs contribute up from 72% to 80% to the total calculated human intake of PAHs. However, the RfDs for these PAHs are not exceeded (table 3). The margins between calculated intake and the RfDs are over a factor 1000. In contrast, for BaP, the only carcinogenic PAH for which a VSD has been derived, the calculated intake exceeds the US-EPA VSD. The RIVM VSD for BaP of 0,5 ng/kg bw/day is not exceeded.

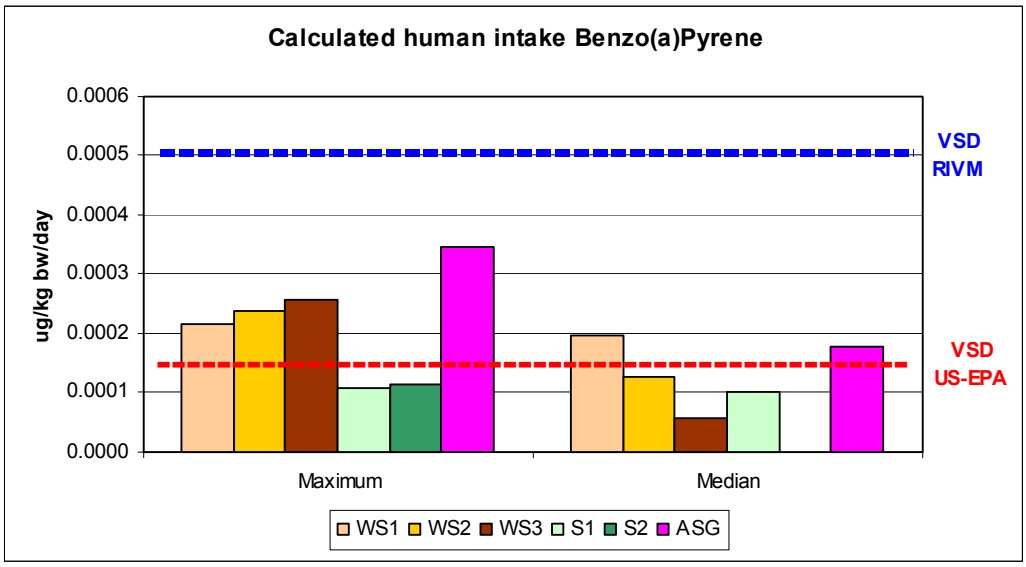


Figure 3. Calculated total human intake of benzo(a)pyrene, based on maximum and median concentrations of PAHs in animal feed ingredients. *Intake in µg/kg bw/day. For comparison, the 'virtually safe dose' for benzo(a)pyrene is also presented.*

Worst case scenario winter 1					
	ACE	**PHE**	**FLU**	**PYR**	**BAP**
RfD	60	40	40	30	0.00014*
Intake human	0.0020	0.0106	0.0018	0.0027	0.0002
Ratio RfD/intake human	-29413	-3760	-22381	-10994	1.55
Worst case scenario winter 2					
	ACE	**PHE**	**FLU**	**PYR**	**BAP**
RfD	60	40	40	30	0.00014*
Intake human	0.0029	0.0242	0.0180	0.0128	0.0002
Ratio RfD/intake human	-20378	-1650	-2225	-2342	1.70
Worst case scenario winter 3					
	ACE	**PHE**	**FLU**	**PYR**	**BAP**
RfD	60	40	40	30	0.00014*
Intake human	0.0038	0.0378	0.0342	0.0229	0.0003
Ratio RfD/intake human	-15590	-1057	-1170	-1311	1.85
Worst case scenario summer 1					
	ACE	**PHE**	**FLU**	**PYR**	**BAP**
RfD	60	40	40	30	0.00014*
Ratio human	0.0006	0.0035	0.0007	0.0009	0.0001
Factor RfD/intake human	-92584	-11455	-59685	-33087	-0.77
Worst case scenario summer 2					
	ACE	**PHE**	**FLU**	**PYR**	**BAP**
RfD	60	40	40	30	0.00014*
Ratio human	0.0007	0.0116	0.0032	0.0053	0.0001
Factor RfD/intake human	-86030	-3448	-12491	-5659	-0.82
Scenario Kees ASG					
	ACE	**PHE**	**FLU**	**PYR**	**BAP**
RfD	60	40	40	30	0.00014*
Ratio human	0.0025	0.0320	0.0345	0.0218	0.0004
Factor RfD/intake human	-24162	-1251	-1160	-1376	2.76

Table 3. Overview of calculated intakes of Benzo(a)pyrene by humans and comparison to the reference values. *Only PAHs for which a reference limit is derived are presented. Reference values and intakes in µg/kg bw/day. A negative ratio indicates that the intake is below the reference value. Marked ratios indicate that the intake exceeds the reference value. ACE=Acenaphthene, BAP=Benzo[a]pyrene, PHE=Phenanthrene, FLU=Fluoranthene, PYR=Pyrene. * Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$.*

7 DISCUSSION

The Dutch Food Safety Authority (VWA) has the incentive to include certain contaminants in the yearly control program based on risk assessments. The yearly performed control program in animal feed has it legal basis in EU legislation. Up to recent years, poly aromatic hydrocarbons (PAHs) were included in the control program, but there is no legal obligation to monitor PAHs. The current (initial) risk assessment was undertaken to provide material to start the discussion on including PAHs in this control program.

The nature of the toxicological profile of PAHs indicated that a distinction has to be made between the low and high molecular PAHs. The high molecular PAHs, of which BaP is the most studied one with its strongest carcinogenic and mutagenic potency of the PAHs, are generally considered critical for the risk assessment. This is because exposure to PAH in food is almost exclusively to a mixture of PAH which includes genotoxic and carcinogenic PAHs (ATSDR, 1995; SCF, 2002; WHO, 2002,). These considerations are based on the total diet. However focussing on animal products (edible tissues and milk) only the profile of PAHs might be different due to the differential transfer of the individual PAHs. In addition the PAHs are extensively metabolised in vivo (Cavret et al., 2004), there is evidence that the metabolites of the high molecular PAHs are transferred at a higher rate to milk than the original compounds (Lutz et al., 2005). These metabolites are important because the carcinogenic potency of the high molecular PAHs can be attributed (at least partly) to these metabolites (ATSDR, 1995).

Whereas the transfer of the high molecular PAHs to edible tissues is generally low, transfer of the low molecular PAHs from animal feed to milk (and other edible tissues) will occur. This is illustrated by the results from the study of Kan et al (2003) indicating that the low molecular PAHs do carry over to milk. The thus obtained transfer factors are influenced by the study design and should be considered carefully, but are nevertheless useful in combination with data from other studies by Grova et al. (2001) and Lutz et al. (2005). Interestingly Lutz et al (2005) included the metabolites in their analysis. Further research on transfer factors and the formation and carry-over of metabolites is clearly needed.

The PAH concentration in animal feed as obtained by the control program in animal feed revealed that a usual pattern of PAHs was present in the feed commodities, e.g. the concentration of low-molecular PAHs was higher than the high-molecular PAHs. To (roughly) estimate the human intake of PAHs via milk some exposure scenario's of lactating cows were calculated. In these scenario's PAH concentrations in feed commodities were obtained from the control program and some additional literature sources. Feed consumption data were taken from a review by Raamsdonk et al (in preparation). Transfer of PAH was estimated based on the study by Kan et al (2003) and relevant literature (Grova et al 2001; Lutz et al 2005).

The scenarios were designed according to the model developed by Raamsdonk et al. (2004). The designs varied reflecting the effect of the use of different feed ingredients in different seasons with different levels of PAH contamination. The impact of feeding of relatively high contaminated grass pellets in feeding regimes was evaluated in the winter scenarios. Additionally, the contribution of fresh grass was evaluated in the summer scenarios. Calculating these scenario's emphasises the need for a complete data set. For example, no data on PAH content of fresh grass were available, data from literature was used instead. For silage only two samples of grass silage were analysed in the control program, whereas grass and corn silage are an important part of the animals diet. Thus, absence of a

complete dataset on PAH concentrations which proportionally covers all feeding ingredients is severely hampering the use of the monitoring data from the control program. If future data from monitoring programs like these should be more suitable for risk assessment, the design of these programs should be changed drastically.

To estimate the human exposure the milk consumption figures from EMEA (standard food basket for the safety assessment of veterinary medicinal products) were used. The standard 1.5 liters of milk consumed on a daily basis are worst case, but come close to the high intake (large portion size) by some Dutch consumers (1.1 liters VCP 97/98).

Based on maximum concentrations, the outcome of the scenarios showed higher variety in outcome than based on median concentrations, with winter scenario 3 resulting in the highest calculated total intake of PAHs. The difference in outcome between scenarios based on maximum and median PAH concentrations indicated that the used maximum concentrations indeed provide a worst case scenario.

Inclusion of grass pellets in the feeding regime contributes highly to the calculated total intake of PAHs. The high percentage of fresh grass in summer scenario 2 feeding regime does not result in such high calculated total PAH intake as compared to the winter scenario 3. This is an indication that the high contribution of PAHs from grass pellets could be due to the contamination during the drying process in the production of the pellets. However, this remains to be determined. The scenario ASG also included grass pellets and resulted in the second highest calculated total intake of PAHs. This indicates that scenario ASG is representative for a worst case scenario.

To evaluate the consequence of the lack of data on fresh grass, summer scenarios 1 and 2 were calculated. For summer scenario 2, data on PAH concentrations in non-contaminated grass were used. This resulted in a 3.5 fold higher calculated total intake of PAHs. Risk assessment based only on the available data from the control program would therefore result in largely underestimating the actual PAH intake in summer.

Distribution of the PAHs in aminal products is similar for all scenarios. The PAHs phenanthrene, fluoranthene and pyrene contribute more than 75% to the calculated total intake of PAHs. This distribution shifts towards fluoranthene when more grass pellets are used in the winter scenarios and in scenario ASG. This indicates that from PAHs in grass pellets, fluoranthene contributes the highest to the calculated total PAH concentration in milk. In the summer scenarios, the contribution of pyrene increases for summer scenario 2, indicating that from PAHs in fresh grass, pyrene contributes the highest to the calculated total PAHs concentration in milk.

No information on effects of PAHs on cows could be found in the reviewed literature. Calculated intake of PAHs by cows is 65 to 1000 times higher than the calculated intake by humans (data not shown). Given the shorter lifespan of cows, comparison to the human RfD is not relevant. However, the higher calculated intake could still be of importance. Cancer of the gastro-intestinal tract is seen in cows (personal comment RIKILT pathologist), which could be an indication for carcinogenic potential of compounds present in animal feed.

The margin between the calculated human intake and the RfDs for non-carcinogenic effects are over a factor 1000. Although combination effects of interacting PAHs could occur, the margin is very high and the risk of non-carcinogenic effects from PAHs originating from feed seems to be minimal. In contrast,

the ‘virtually safe dose’ (VSD) of 0.14 ng/kg bw/day for BaP (US-EPA based on carcinogenic risk of $1*10^{-6}$) is exceeded for the winter scenarios (factor 1.85 for WS3) and for scenario ASG (factor 2.75). Based on median concentrations this dose is exceeded for winter scenarios 1 and scenario ASG. The calculated intakes do not exceed the VSD of 0.5 ng/kg bw/day as set by RIVM, either based on maximum or median concentrations. Exceeding the VSD seems contradictory with the data from Kan et al. (2003) where no carcinogenic PAHs were detected in milk above the detection limits of 0.1 ng/g. Calculated BaP levels in the scenario’s were in the range of 4 to 14 ng/l and thus higher than the detection limits. However, the transfer rate used for the calculated human intake was taken from the study of Grova et al. (2002), which showed a transfer of 0.2% BaP related activity from radiolabeled PAHs from feed to milk in goat. Since BaP was not detected in cows milk in other studies, it could be possible that the transfer in cows is different than in goats. BaP metabolites are usually not analysed in milk, so it seems likely that the 0.2% BaP related radioactivity results from transferred BaP metabolites. In this case, it is not clear to what extend the VSD of 0.14 ng/kg bw/day for BaP can be applied. However, the VSD is exceeded for the worst case scenarios, so detailed analysis on occurrence and effects of metabolites of BaP (and other PAHs) is recommended for facilitating more accurate risk assessment.

Notwithstanding the limitations of the initial risk assessment and it (worst case) assumptions it can be concluded that there are good reasons to include PAHs and its metabolites into the control program. To obtain more information on the actual exposure of animals and humans the design of the monitoring schedule needs to be carefully reconsidered. In addition, more information on the transfer of PAHs from feed to animal products is needed.

8 CONCLUSIONS

Toxicology

- Data on non-carcinogenic effects of Poly Aromatic Hydrocarbons (PAHs) are very limited. Similar applies to the oral carcinogenic potential of PAHs other than Benzo(a)Pyrene (BaP).

Scenarios

- The experimental scenario ASG seems representative of a worst case scenario;
- Of the PAHs present in grass pellets, fluoranthene contributes the most to the calculated total PAH concentration in milk;
- Of the PAHs present in fresh grass, pyrene contributes the most to the calculated total PAHs concentration in milk.

Transfer from feed to animal products

- Data on transfer rates of PAHs to cow's milk and other animal products are very limited; Metabolised PAHs are generally not considered in the transfer of PAHs from feed to food. It is likely that PAHs with more than 5 rings are transferred as metabolites;
- Artificially dried roughage feed, such as grass pellets, contribute the most to the total concentration of PAHs in milk.

Human oral exposure

- The calculated human intake of PAHs from animal feed via milk is far below the RfDs set for non-carcinogenic effects;
- Calculated transfer of high-molecular PAHs from feed to cows milk results exceeding the 'virtually safe dose' for BaP up to a factor 2.75 for humans consuming this milk. Based on the data in literature, this could be the result from transfer of BaP metabolites.

Animal oral exposure

- Calculated intake of PAHs by cows is 65 to 1000 times higher than the calculated intake by humans, which could indicate a potential risk for animal health.

Control program

- The current analysis of PAHs in food should not be expressed solely in BaP equivalents;
- Analysis of PAHs in animal products should also include metabolites of PAHs;
- For use of the monitoring results in risk assessment, the design of the control program should be adapted.

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APPENDIX A

Processing of data

PAH concentrations have been measured in several feed ingredients in the years 2000-2004.

1) These ingredients were categorised;
2) Maximums and medians were calculated per category;
3) These maxima were then used to calculate possible PAHs concentrations in mixed feed using the composition of mixed feed as provided by Kemme and Van Raamsdonk (2004);
4) With the calculated maximum concentrations in mixed feed and measured maximum concentrations in rough feed, a maximum PAHs intake for cattle was calculated for several scenarios of feed consumption of cattle;
5) Transfer rates of PAHs from feed to milk were taken from literature. In addition, transfer rates for native PAHs are calculated from the study of Kan et al. (2004);
6) Using these transfer rates, maximum PAH concentrations in milk were calculated for several scenarios;
7) Human intake is calculated using the standard food basket (1.5 l milk/day) from EMEA;
8) Calculated human intake is compared to the known toxicological reference values.

Please note that all data is standardised for 12% water content of the product

Ad 1- Categories differ in number of samples analysed (see table A1).

Category	**Matrix**	**# samples (n)**
1-Flakes	copra extraction pellets	8
2-Oils/fats	animal fat	15
	destruction fat	9
	frying fat	5
	linolic fatty acids	1
	palm oil	2
	palm oil fatty acids	1
	vegetable oil/fat	8
	poultry fat	2
	soy oil	5
	tall oil sterols	27
	pork fat	1
3-Citrus	citrus pulp pellets	9
4-Roughage dried	dried grass	1
	grass pellets	68
	lucerne pellets	39
4a-Roughage silage	grass silage	2
5-Other	potatoe	3
	beet	2
	beans	2
	cabbage	2
	lettuce	1
	onion	1

Table A1*. Specification of feed ingredients per category.*

Ad 2- Maxima and medians of PAH concentrations measured in the feed ingredients in the Control program animal feed are specified in tables A2 and A3.

Category		ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR
1	**Flakes**	34.78	2.00	3.17	2.00	1.74	0.38	4.79	0.14	76.16	15.83	1.10	31.87
2	**Oils/fats**	9.20	6.50	7.60	9.80	9.50	7.20	9.00	6.30	9.00	9.60	9.10	9.50
3	**Citrus**	5.30	2.50	7.60	9.60	6.50	2.40	9.20	0.10	0.10	0.10	6.90	0.10
4	**Roughage dried**	13.36	12.94	14.30	9.80	10.18	8.80	15.07	8.40	80.80	157.40	8.50	98.20
4a	**Roughage silage**	1.20	1.80	8.20	7.90	9.10	4.30	0.72	1.20	3.90	2.70	5.40	1.90
5	**Other**	1.10	0.66	6.00	7.30	6.00	3.10	5.30	0.88	5.10	4.60	5.00	2.90

***Table A2**. Maximum concentrations PAHs in feed categories in µg/kg. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene. In citrus, no usable data was obtained for DBA, PHE, FLU and PYR due to conversion errors from Gas chromatograph. For these PAHs, the detection limit was taken as maximum concentration.*

Category		ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR
1	**Flakes**	6.03	1.78	2.11	0.99	0.97	0.28	2.53	0.13	67.72	14.49	0.83	19.52
2	**Oils/fats**	2.95	2.50	1.90	1.50	1.40	0.65	2.30	0.99	3.40	4.50	1.15	7.45
3	**Citrus**	4.20	2.05	5.75	5.55	5.20	1.85	9.15	0.00	0.00	0.00	4.45	0.00
4	**Roughage dried**	0.89	1.35	1.60	1.60	1.95	1.40	2.90	1.20	5.40	4.60	1.70	3.75
4a	**Roughage silage**	1.00	1.80	8.20	7.90	4.92	4.30	0.72	1.20	3.90	2.70	5.40	1.90
5	**Other**	0.89	0.20	0.61	0.29	0.36	0.40	0.25	0.52	2.00	0.58	0.27	0.44

***Table A3.** Median concentrations PAHs in feed categories in µg/kg. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.*

In the control program no fresh grass samples were analysed. However fresh grass is a major part of the consumption of roughage by cows during summer. Therefore from literature, PAH concentrations in fresh grass were taken. These were applied in Summer scenario 2 (see Ad 4). PAH concentrations in fresh grass were taken from Crépineau-Ducoulombier et al. (2004, control site 2), where concentrations lower than detection limit were assumed to be at the level of detection of 0.50 µg/kg.

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR
Fresh grass	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	34.54	18.22	0.50	31.62

***Table A4**. PAH concentrations in fresh grass in µg/kg. Data taken from Crépineau-Ducoulombier et al. (2004, control site 2). Concentrations lower than detection limit were assumed to be at the level of detection of 0.50 µg/kg. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.*

Ad 3- Contribution of individual feed ingredient categories to compound feed of cattle (Kemme en Van Raamsdonk, 2004). Not all ingredients for compound feed were represented in the samples from the Control program, explaining why the sum of the percentages of contribution of individual feed ingredient categories is less than 100%. It may be possible that PAHs in the ingredients not represented here might have added to the calculated PAH content of compound feed. For this initial risk assessment it is assumed that all PAHs in the concentrate originate only from the feed ingredients listed in table A5.

Category		Average %
1	Flakes	57
2	Oils/fats	0.4
3	Citrus	3.5
4	Roughage	0.4
5	Other	1.6

Table A5*. Contribution of feed ingredient categories to compound feed of cattle (from Kemme en Van Raamsdonk, 2004)*

Ad 4- Specification of the applied feeding regimes in the scenarios used in this report. The PAH concentration in corn silage was assumed to be the same as in grass silage, thus corn and grass silage were used as one feeding category. Scenarios were derived from Van Raamsdonk et al., 2006 (in preparation). Results from the scenarios are presented in Appendices B-G.

	Compound feed	Roughage-silage	Roughage-dried	Fresh grass
Winter scenario 1	7.1	11.9	0	0
Winter scenario 2	7.1	5.95	5.95	0
Winter scenario 3	7.1	0	11.9	0
Summer scenario 1	2.1	6.5	0	0*
Summer scenario 2	2.1	6.5	0	7.9
Scenario ASG	1	11.3	10	0

Table A6. *Applied feeding regimes in the scenarios used for risk assessment****.*** *Feed is in kg per cow per day. * lack of PAH monitoring data is represented as 0 kg of grass consumption.*

Ad 5- Transfer rates of PAHs from feed to milk were taken from literature (see table A4). Lutz et al. (2005) measured the concentration of native PAHs and their metabolites in milk of cows which were orally exposed to soil which contained PAHs. They found no transfer of native PAHs but did find metabolites in the milk. Grova et al, (2002) measured PAH related C14 activity in milk after oral exposure of goats to several labeled PAHs. No distinction was made between native compounds or their metabolites, so the measured activity can be related to both.

In addition, transfer rates for native PAHs are calculated from the study of Kan et al. (2004). For this, the total amount of PAHs consumed by the cows was calculated and compared to the measured concentrations in milk. In this comparison, the assumption was made that all measured PAHs originated from the PAH in the contaminated feed. This results in an overestimation of the transfer rate, because background concentrations in the milk are neglected. No metabolites were measured in this study, so transfer rates are limited to parent compounds.

		ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR
Lutz 2005	Native PAH				0					0			0
	Metabolite				0					0.03			1.62
Grova 2002	PAH related C14				0.20					1.60			1.90
Kan 2003	Native PAH Metabolites not done	1.35						0.05		3.21	0.44		0.39
Applied in this Risk Assessment		1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90

Table A4. *Transfer rates of PAHs from feed to milk in percentages. Data taken from literature and calculation. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.*

Using these transfer rates, maximum PAH concentrations in milk were calculated. For PAHs for which no transfer rates were available, the maximum of transfer rates for low- and high molecular PAHs was applied. Although pyrene was not classified as low-molecular PAH, its transfer rate (1.9%) was taken for this group because of similar transfer behaviour to the low-molecular PAHs. For high-molecular PAHs this was 0.2% (from Benzo(a)pyrene). Because of use of the total transfer rates from the PAH related activity in the study of Grova, no distinction was made for transfer of native compounds or metabolites in the evaluation.

Ad 6- The milk production corresponding with the food regimes used in the scenarios was 27 liters for the winter- and summer scenarios (Van Raamsdonk et al., 2004), and 25 liters for the ASG scenario (Kan et al., 2003). Results from calculated transfer are summarized in the results from the scenarios in Appendix B-G.

Ad 7- Human PAH intake from milk is calculated using the standards for milk consumption (1.5 liters) and body weight (60 kg) as set by EMEA.

APPENDIX B

Winter scenario 1

Applied feeding regime in kg per cow per day.

Compound feed	7.1
Roughage dried	0
Roughage silage	11.9

Three winter scenarios were designed because of the relative high levels of PAHs in artificially dried grass pellets. The winter feeding regime as used by Van Raamsdonk et al. (2006, in preparation) for milk production of 27 liters was used as a basis for the winterscenarios. The amount of 0.97 kg moist compound feed as defined in the winter feeding regime of Van Raamsdonk et al. was not taken up in the winterscenarios because of lack of data from the Control program for this feeding categorie.
In the winterscenarios, the percentage of silage in the winter feeding regime is replaced in varying proportions by grass pellets to simulate this variety in feed sources. In this first scenario, all roughage is assumed to consist of silage.

Results from calculation

In the tables below an overview is given of calculated transfer of PAHs from feed to milk and calculated intakes by humans and cows. In addtion, the factor between the reference value and the calculated intake is given. A negative factor indicates that the intake is below the reference value. Yellow marked factors indicate that the intake exceeds the reference value.

* Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

Results based on maximum PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	163.19	33.63	122.35	116.92	125.65	56.87	42.11	15.48	357.92	101.59	79.41	155.10	1370.23
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	2.20	0.64	0.24	0.23	0.25	0.11	0.02	0.03	11.49	1.93	0.16	2.95	20.26
ug/l PAH in milk (27l)	0.08	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.43	0.07	0.01	0.11	0.75
Human intake (1.5 l milk)	0.122	0.036	0.014	0.013	0.014	0.006	0.001	0.002	0.638	0.107	0.009	0.164	1.13
ug/kg bw (60 kg EMEA)	0.002	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.002	0.000	0.003	0.02
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	
Ratio human intake/RfD	-29413			1.55					-3760	-22381		-10994	

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	42.38	31.63	114.34	105.91	69.83	55.00	31.80	14.91	320.93	91.11	73.94	101.97	1053.74
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	0.57	0.60	0.23	0.21	0.14	0.11	0.02	0.03	10.30	1.73	0.15	1.94	16.03
ug/l PAH in milk (27l)	0.02	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.38	0.06	0.01	0.07	0.59
Human intake (1.5 l milk)	0.032	0.033	0.013	0.012	0.008	0.006	0.001	0.002	0.572	0.096	0.008	0.108	0.89
ug/kg bw (60 kg EMEA)	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.002	0.000	0.002	0.02
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	
Ratio human intake/RfD	-113252			1.40					-4193	-24957		-16724	

APPENDIX C

Winter scenario 2

Applied feeding regime in kg per cow per day.

Compound feed	7.1
Roughage dried	5.95
Roughage silage	5.95

Three winter scenarios were designed because of the relative high levels of PAHs in artificially dried grass pellets. The winter feeding regime as used by Van Raamsdonk et al. (2006, in preparation) for milk production of 27 liters was used as a basis for the winterscenarios. The amount of 0.97 kg moist compound feed as defined in the winter feeding regime of Van Raamsdonk et al. was not taken up in the winterscenarios because of lack of data from the Control program for this feeding categorie.
In the winterscenarios, the percentage of silage in the winter feeding regime is replaced in varying proportions by grass pellets to simulate this variety in feed sources. In this second scenario, roughage is assumed to consist of half grass pellets and half of silage.

Results from calculation
In the tables below an overview is given of calculated transfer of PAHs from feed to milk and calculated intakes by humans and cows. In addtion, the factor between the reference value and the calculated intake is given. A negative factor indicates that the intake is below the reference value. Yellow marked factors indicate that the intake exceeds the reference value.
* Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

Results based on maximum PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	235.55	99.92	158.65	128.23	132.08	83.64	127.48	58.32	815.47	1022.06	97.86	728.09	3687.34
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	3.18	1.90	0.32	0.26	0.26	0.17	0.06	0.12	26.18	19.42	0.20	13.83	65.89
ug/l PAH in milk (27l)	0.12	0.07	0.01	0.01	0.01	0.01	0.00	0.00	0.97	0.72	0.01	0.51	2.44
Human intake (1.5 l milk)	0.177	0.105	0.018	0.014	0.015	0.009	0.004	0.006	1.454	1.079	0.011	0.769	3.66
ug/kg bw (60 kg EMEA)	0.003	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.018	0.000	0.013	0.06
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-20378	-	-	1.70	-	-	-	-	-1650	-2225	-	-2342	-

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	41.73	28.95	75.07	68.42	52.19	37.74	44.77	14.91	329.85	102.41	51.93	112.97	960.95
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	0.56	0.55	0.15	0.14	0.10	0.08	0.02	0.03	10.59	1.95	0.10	2.15	16.42
ug/l PAH in milk (27l)	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.39	0.07	0.00	0.08	0.61
Human intake (1.5 l milk)	0.031	0.031	0.008	0.008	0.006	0.004	0.001	0.002	0.588	0.108	0.006	0.119	0.91
ug/kg bw (60 kg EMEA)	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.002	0.000	0.002	0.02
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-115028	-	-	0.91	-	-	-	-	-4080	-22202	-	-15094	-

APPENDIX D

Winter scenario 3

Applied feeding regime in kg per cow per day.

Compound feed	7.1
Roughage dried	11.9
Roughage silage	0

Three winter scenarios were designed because of the relative high levels of PAHs in artificially dried grass pellets. The winter feeding regime as used by Van Raamsdonk et al. (2006, in preparation) for milk production of 27 liters was used as a basis for the winterscenarios. The amount of 0.97 kg moist compound feed as defined in the winter feeding regime of Van Raamsdonk et al. was not taken up in the winterscenarios because of lack of data from the Control program for this feeding categorie.
In the winterscenarios, the percentage of silage in the winter feeding regime is replaced in varying proportions by grass pellets to simulate this variety in feed sources. In this third scenario, all roughage is assumed to consist of grass pellets.

Results from calculation
In the tables below an overview is given of calculated transfer of PAHs from feed to milk and calculated intakes by humans and cows. In addtion, the factor between the reference value and the calculated intake is given. A negative factor indicates that the intake is below the reference value. Yellow marked factors indicate that the intake exceeds the reference value.
* Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

Results based on maximum PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	307.90	166.20	194.94	139.53	138.51	110.42	212.85	101.16	1273.03	1942.52	116.30	1301.07	6004.44
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	4.16	3.16	0.39	0.28	0.28	0.22	0.11	0.20	40.86	36.91	0.23	24.72	111.51
ug/l PAH in milk (27l)	0.15	0.12	0.01	0.01	0.01	0.01	0.00	0.01	1.51	1.37	0.01	0.92	4.13
Human intake (1.5 l milk)	0.231	0.175	0.022	0.016	0.015	0.012	0.006	0.011	2.270	2.050	0.013	1.373	6.20
ug/kg bw (60 kg EMEA)	0.004	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.034	0.000	0.023	0.10
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-15590	-	-	1.85	-	-	-	-	-1057	-1170	-	-1311	-

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	41.07	26.27	35.80	30.94	34.55	20.49	57.74	14.91	338.78	113.72	29.91	123.98	868.16
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	0.55	0.50	0.07	0.06	0.07	0.04	0.03	0.03	10.87	2.16	0.06	2.36	16.81
ug/l PAH in milk (27l)	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.08	0.00	0.09	0.62
Human intake (1.5 l milk)	0.031	0.028	0.004	0.003	0.004	0.002	0.002	0.002	0.604	0.120	0.003	0.131	0.93
ug/kg bw (60 kg EMEA)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.002	0.000	0.002	0.02
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-116861	-	-	0.41	-	-	-	-	-3972	-19995	-	-13754	-

APPENDIX E

Summer scenario 1

Applied feeding regime in kg per cow per day.

Compound feed	2.1
Grass	0
Roughage silage	6.5

Two summerscenarios were designed because of the lack of data from fresh grass samples in the Control program. The summer feeding regime as used by Van Raamsdonk et al. (2006, in preparation) for milk production of 27 liters was used as a basis for the summerscenarios. The amount of 0.26 kg moist compound feed as defined in the summer feeding regime of Van Raamsdonk et al. was not taken up in the summerscenarios because of lack of data from the Control program for this feeding categorie.The scenarios differ in the use of PAH concentrations in fresh grass, for one scenario no data are used (current situation Control program), and for one scenario data is taken from literature. In this first summerscenario, no data for fresh grass were used, represented as consumption of 0 kg in the table above.

Results from calculation

In the tables below an overview is given of calculated transfer of PAHs from feed to milk and calculated intakes by humans and cows. In addtion, the factor between the reference value and the calculated intake is given. A negative factor indicates that the intake is below the reference value. Yellow marked factors indicate that the intake exceeds the reference value.

* Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

Results based on maximum PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	51.84	15.31	60.63	58.13	64.28	29.64	14.60	8.16	117.49	38.09	39.58	51.54	549.29
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	0.00
PAHs transferred (in ug)	0.70	0.29	0.12	0.12	0.13	0.06	0.01	0.02	3.77	0.72	0.08	0.98	6.99
ug/l PAH in milk (27l)	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.03	0.00	0.04	0.26
Human intake (1.5 l milk)	0.039	0.016	0.007	0.006	0.007	0.003	0.000	0.001	0.210	0.040	0.004	0.054	0.39
ug/kg bw (60 kg EMEA)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.000	0.001	0.01
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	92584	-	-	0.77	-	-	-	-	11455	59685	-	33087	-

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	15.52	14.72	58.26	54.87	35.30	29.08	11.55	7.99	106.55	34.99	37.96	35.82	442.61
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	0.21	0.28	0.12	0.11	0.07	0.06	0.01	0.02	3.42	0.66	0.08	0.68	5.71
ug/l PAH in milk (27l)	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.02	0.00	0.03	0.21
Human intake (1.5 l milk)	0.012	0.016	0.006	0.006	0.004	0.003	0.000	0.001	0.190	0.037	0.004	0.038	0.32
ug/kg bw (60 kg EMEA)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.000	0.001	0.01
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	309353	-	-	0.73	-	-	-	-	12631	64975	-	47605	-

APPENDIX F

Summer scenario 2

Applied feeding regime in kg per cow per day.

Compound feed	2.1
Grass	7.9
Roughage silage	6.5

Two summerscenarios were designed because of the lack of data from fresh grass samples in the Control program. The summer feeding regime as used by Van Raamsdonk et al. (2006, in preparation) for milk production of 27 liters was used as a basis for the summerscenarios. The amount of 0.26 kg moist compound feed as defined in the summer feeding regime of Van Raamsdonk et al. was not taken up in the summerscenarios because of lack of data from the Control program for this feeding categorie.The scenarios differ in the use of PAH concentrations in fresh grass, for one scenario no data are used (current situation Control program), and for one scenario data is taken from literature. In this second summer scenario, data from fresh grass were used from the study of Crépineau-Ducoulombier et al. (2004, control site 2).

Results from calculation

In the tables below an overview is given of calculated transfer of PAHs from feed to milk and calculated intakes by humans and cows. In addtion, the factor between the reference value and the calculated intake is given. A negative factor indicates that the intake is below the reference value. Yellow marked factors indicate that the intake exceeds the reference value.

* Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

Results based on maximum PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	51.84	15.31	60.63	58.13	64.28	29.64	14.60	8.16	117.49	38.09	39.58	51.54	549.29
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	0.00
PAHs transferred (in ug)	0.70	0.29	0.12	0.12	0.13	0.06	0.01	0.02	3.77	0.72	0.08	0.98	6.99
ug/l PAH in milk (27l)	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.03	0.00	0.04	0.26
Human intake (1.5 l milk)	0.039	0.016	0.007	0.006	0.007	0.003	0.000	0.001	0.210	0.040	0.004	0.054	0.39
ug/kg bw (60 kg EMEA)	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.000	0.001	0.01
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-92584	-	-	0.77	-	-	-	-	-11455	-59685	-	-33087	-

Because data for fresh grass are taken from literature, no median concentrations were available for summer scenario 2. Using median concentrations of 0 µg/kg as an alternative would result in the same calculation as summer scenario 1 and is therefore not performed.

APPENDIX G

Scenario ASG

Applied feeding regime in kg per cow per day.

Compound feed	1
Roughage dried	11.3
Roughage silage	10

The feeding regime as used in the study of Kan et al. (2003) was used with the data from the Control program. The feeding regime was selected by Kan et al. to contain the highest possible amount of contaminated feed, in order to facilitate a maximum transfer rate of PAHs from the feed to milk.

Results from calculation

In the tables below an overview is given of calculated transfer of PAHs from feed to milk and calculated intakes by humans and cows. In addtion, the factor between the reference value and the calculated intake is given. A negative factor indicates that the intake is below the reference value. Yellow marked factors indicate that the intake exceeds the reference value.

* Value is not an RfD but a 'virtually safe dose' calculated by US-EPA based on carcinogenic risk of $1*10^{-6}$. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene, PHE=Phenanthrene, FLU=Fluoranthene, IDP=Indeno[1,2,3-c,d]pyrene, PYR=Pyrene.

Results based on maximum PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	183.94	165.94	247.08	192.97	208.49	143.24	182.19	107.09	995.91	1815.40	152.18	1147.32	5541.77
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	2.48	3.15	0.49	0.39	0.42	0.29	0.09	0.21	31.97	34.49	0.30	21.80	96.09
ug/l PAH in milk (27l)	0.10	0.13	0.02	0.02	0.02	0.01	0.00	0.01	1.28	1.38	0.01	0.87	3.84
Human intake (1.5 l milk)	0.149	0.189	0.030	0.023	0.025	0.017	0.005	0.013	1.918	2.070	0.018	1.308	5.77
ug/kg bw (60 kg EMEA)	0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.034	0.000	0.022	0.10
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-24162	-	-	2.76	-	-	-	-	-1251	-1160	-	-1376	-

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total
PAH intake cow (in ug)	24.35	34.69	102.44	98.76	72.78	59.36	43.24	25.65	138.68	87.29	74.57	72.55	834.37
Used transfer in %	1.35	1.90	0.20	0.20	0.20	0.20	0.05	0.20	3.21	1.90	0.20	1.90	11.51
PAHs transferred (in ug)	0.33	0.66	0.20	0.20	0.15	0.12	0.02	0.05	4.45	1.66	0.15	1.38	9.37
ug/l PAH in milk (27l)	0.01	0.03	0.01	0.01	0.01	0.00	0.00	0.00	0.18	0.07	0.01	0.06	0.37
Human intake (1.5 l milk)	0.020	0.040	0.012	0.012	0.009	0.007	0.001	0.003	0.267	0.100	0.009	0.083	0.56
ug/kg bw (60 kg EMEA)	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.001	0.01
RfD ug/kg bw/day	60	-	-	0.00014*	-	-	-	-	40	40	-	30	-
Ratio human intake/RfD	-182520	-	-	1.41	-	-	-	-	-8985	-24119	-	-21763	-