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Risk assessment of Nickel, Mineral Oils, Polycyclic Aromatic Hydrocarbons and Volatile Organic Compounds in animal feed materials

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Executive summary

In this report it is evaluated whether nickel, mineral oil, poly aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) should be included in the National Control Plan for Animal Feed. To date, no maximum permitted levels have been established for these (groups of) compounds, so there is no regulatory basis for inclusion of the compounds in the monitoring. To evaluate whether inclusion is necessary to ensure safety of food producing animals and consumers of animal products, risk assessments were performed. For this, toxicological profiles of the compounds were made for hazard characterization, and subsequently data from the National Control Plan for Animal Feed (years 2000-2004) were used for scenario calculations to estimate the potential risks. For PAHs, additional information on PAH carry-over from the report 'Voorkomen van PAK's in voer, omgeving van dieren, melk en zuivelproducten alsmede een oriënterende studie in melkvee' of Kan et al (2003) was used. The risk assessments are presented per (group of) compound(s) in this report.

The recommendations as regards to inclusion of compounds in the National Control Plan for Animal Feed are:

- *Not to include nickel, mineral oils or VOCs in the National Control Plan for Animal Feed.*
- *To include PAHs in the National Control Plan for Animal Feed*

From the risk assessments, the following points of interest can be summarized:

Nickel in mineral supplements

Nickel is used in food production as a catalyzing agent in vegetable oil refinery. Residues of this use in food are not expected to pose a risk to consumers. The total daily oral intake has been estimated by EFSA (2005) to be at the level of the lowest TDI found. The predominant oral nickel exposure of humans can be mainly attributed to the consumption of plants contaminated with nickel. Since the expected contribution of nickel originating from animal feed to the daily intake by consumers was expected to be very low, no further risk assessment was performed. The possible risk to animal health could not be estimated because no information was available on the amounts of mineral feed supplements used in animal husbandry.

Recommendations:

- *To gather information on the use of mineral feed supplements in animal husbandry.*

Mineral oils

Food grade mineral oils, or hydrocarbons, are produced as by-products during the refining and distillation of crude mineral oils. In practice, several definitions for mineral oils are used. For this risk assessment, only the aliphatic (straight) alkanes with carbon numbers between 10 and 40 were considered. The risk assessment was severely hampered by the fact that the TDIs for mineral oils are specified for the separate alkane fractions, while the results of the analysis of hydrocarbons in food or feed are usually expressed as (general) alkanes.

The main toxicological effect following oral exposure was the appearance of microgranoluma in mesenterial lymph nodes, but only in one species of rats. Similar phenomena have been observed in humans after oral exposure to mineral oils. Much controversy on the subject of the toxicological relevance of this effect exists. The implication of oral exposure to mineral oils for human health could therefore not be determined.

It was found that alkanes may carry over to meat, contrary to the general assumption that alkanes are not transferred from feed to animal products. Intake assessment indicated that risks for animal health could not be excluded.
Recommendations:

- *To repeat the risk assessment if new information indicating the relevance of the effects of exposure to mineral oil on mesenterial lymph nodes to human health becomes available.*

Polyaromatic hydrocarbons (PAHs)

Animal feed materials may contain elevated concentrations of PAHs due to environmental contamination or through direct drying processes. 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 aromatic rings. Furthermore, there is evidence that indicate that metabolites of some PAHs are transferred to milk, which is of interest because the carcinogenic properties of PAHs can be contributed to the PAH metabolites. Several (worst case) exposure scenarios were calculated, using the maximum measured PAH concentrations from the National Control Plan for Animal Feed and (limited) carry-over rates available from literature. The results indicate that the intake of PAHs by cattle can be in the mg-range. Calculated intake of PAHs by humans via milk is at least a factor 1000 under the chronic Reference Dose (RfD) for non-carcinogenic effects. In contrast, calculated intake by humans of high-molecular (carcinogenic) PAHs calculated to be present in milk exceeded the 'virtually safe dose' for Benzo(a)Pyrene in several worst case scenarios. Although the scenarios are worst case in regard to the use of maximum measured concentrations, not all feed ingredient categories were represented in the dataset. This incomplete calculation results in this way in underestimating the intake assessment of PAHs.
The occurrence of metabolites of PAHs has not been considered in monitoring programs of animal products yet. Considering their expected carcinogenicity, this is an urgent matter of attention.
Recommendations:

- *To gather more information on carry-over of PAHs and their metabolites from feed to milk and other animal products*
- *To incorporate toxicity of possible transferred (toxic) metabolites in risk assessment*
- *To include feed ingredient categories in the monitoring of animal feed which contribute largely to the feeding regime.*

Volatile organic compounds (VOCs)

VOCs are simple hydrocarbons that originate from crude oil and derived products. In food production, they are mainly used as solvents for extracting oil from oilseeds, which may result in residues in food and feed ingredients. Very little oral toxicity data are available on these compounds. A worst case scenario using maximum measured concentrations of VOCs (which will be reduced during processing of ingredients to compound feed) and 100% (unrealistic) carry-over from feed to animal products does not result in calculated intake by humans above the known reference limits. The scenario is overestimating exposure in view of the expectation that the concentrations of VOCs are reduced during processing of ingredients to compound feed and that 100% transfer is unrealistic. However, not all feed ingredient categories were sampled and could not be included in the calculation.

It is not known to what extent (toxic) metabolites of VOCs are formed and carried over to animal products, therefore the risk from intake of metabolites could not be excluded. A quantitative intake assessment for food producing animals indicated that animal health effects may occur.
Recommendations:

- *To gather data on VOC concentrations in compound feed end product in order to perform a new risk assessment aimed at animal health*
- *For risk assessment purposes, gather more information on formation and carry-over of toxic VOC metabolites from feed to milk and other animal products*

General

- From the risk assessments of PAHs and VOCs it can be concluded that carry-over of metabolites of compounds present in animal feed to animal products needs attention. Usually, only parent compounds are monitored in animal products.
- In monitoring more attention should be given to the amounts of (types of) feed consumed by the animal. Feed ingredient categories which are not regarded as 'risk materials' for certain compounds but cover a large part of the total feeding regime, may still add significantly to the total intake of contaminants. For accurate risk assessment, concentrations in these ingredients should be known.
- As defined by regulation 1831/2003, processing aids are allowed to be used in feed production as long as residues in feed do no pose a risk for animals or humans. As shown in the case of VOCs, oral toxicity data may not be available to prove the lack of toxic effects. This may result in undesirable compounds to be used in feed production. As regards processing aids, the question may therefore be posed if the Regulation is fully effective in protecting human and/or animal health.

Samenvatting

In dit rapport is geëvalueerd of nikkel, minerale olie, polyaromatische koolwaterstoffen (PAKs) en vluchtige organische verbindingen (VOCs) opgenomen zouden moeten worden in het Nationaal Plan voor contaminanten in diervoeder. Omdat er tot op heden geen maximum limieten zijn vastgesteld voor deze (groepen van) stoffen, ontbreekt de wettelijke basis hiervoor. Om te bepalen of monitoring van deze stoffen nodig is voor de bescherming van de gezondheid van landbouwhuisdieren en consumenten van dierlijke producten, zijn voor de stoffen risicobeoordelingen uitgevoerd. Voor het bepalen van mogelijke effecten zijn er toxicologische profielen opgesteld, op basis waarvan bepaald is of het uitvoeren van een oriënterende risicobeoordeling met data uit het Nationaal Plan voor Diervoeders zinvol is. Hiervoor zijn data gebruikt uit de jaren 2000-2004. Voor PAKs zijn er aanvullende data met betrekking tot overdracht gebruikt uit het rapport 'Voorkomen van PAKs in voer, omgeving van dieren, melk en zuivelproducten alsmede een oriënterende studie in melkvee' van Kan et al. (2003). De risicobeoordelingen zijn in dit rapport weergegeven per (groep van) stof(fen).

De aanbevelingen ten aanzien van het opnemen van de stoffen in het Nationaal Plan voor Diervoeders zijn:

- *Het niet opnemen van nikkel, mineral olie of VOCs in het Nationaal Plan voor Diervoeders*
- *Het opnemen van PAKs in het Nationaal Plan voor Diervoeders*

Uit de risicobeoordeling komen de volgende aandachtspunten naar voren:

Nikkel in mineralenmengsels

In voedselproductie wordt nikkel gebruikt als katalysator in de raffinage van plantaardige vetten. Het is niet te verwachten dat residuen uit dit proces een risico vormen voor de consument. De totale dagelijkse inname is in 2005 door EFSA geschat op het niveau van de laagst gevonden grenswaarde. In eerdere jaren is nikkel in hoge gehalten aangetroffen in mineralenmengsels voor diervoeders. Echter wordt de grootste bron van inname van nikkel door de mens veroorzaakt door ophoping van nikkel in eetbare gewassen. Aangezien de bijdrage van nikkel in diervoeding via dierlijke producten aan de dagelijkse inname van consumenten naar verwachting erg laag is, is er geen verdere risicobeoordeling uitgevoerd voor de mens. Voor landbouwhuisdieren zou het risico anders kunnen liggen. Het risico voor de diergezondheid kon echter niet worden geschat omdat er geen gebruiksgegevens over mineralenmengsels beschikbaar waren.

Aanbevelingen:

- *Het verzamelen van gebruiksgegevens van mineralenmengsels, zodat een betrouwbare risicobeoordeling van mogelijke contaminanten in de mengsels kan worden uitgevoerd.*

Minerale olie

Minerale olie voor gebruik in voedsel, ook wel alkanen genoemd, worden geproduceerd als bijproducten tijdens het raffineren en destilleren van ruwe minerale olie. In de praktijk worden er verschillende definities voor minerale olie gebruikt. Voor deze risicobeoordeling worden met minerale olie enkel de rechte (alifatische) alkanen met ketens van 10 tot 40 C atomen bedoeld. De risicobeoordeling werd ernstig bemoeilijkt door het feit dat de toxicologische grenswaarden voor minerale olie gespecificeerd voor verschillende alkaanfracties, terwijl analyseresultaten waren gegeven in het totale gehalte aan alkanen.

Het voornaamste toxicologische effect na orale inname, is het verschijnen van microgranuloma in de mesenteriale lymfeklieren, maar slechts in één soort rat. Vergelijkbare verschijnselen zijn geconstateerd in mensen na orale toediening van minerale olie. Tot op heden is er nog geen eenduidige opvatting van de relevantie van dit effect voor de gezondheid. Het risico van minerale olie via inname van dierlijke producten voor de humane gezondheid kon daarom niet worden ingeschat.
Een van de resultaten was dat, in tegenstelling tot wat algemeen wordt aangenomen, de mogelijkheid bestaat dat minerale olie vanuit diervoeder zou kunnen overdragen naar dierlijke producten (vlees). Op basis van berekende inname kunnen daarnaast nadelige effecten op de diergezondheid niet worden uitgesloten.
Aanbevelingen:

- *De risicobeoordeling te herhalen wanneer er nieuwe inzichten zijn met betrekking tot het belang van de effecten van blootstelling aan minerale olie op mesenteriale lymfeklieren*

Polyaromatische koolwaterstoffen (PAKs)
Ingrediënten van diervoeder kunnen verhoogde gehalten aan PAKs bevatten door milieuverontreiniging of door (directe) droogprocessen. Hoewel erg weinig data beschikbaar is over de overdrachtspercentages, wordt er in het algemeen aangenomen dat PAKs niet overdragen vanuit diervoeder naar dierlijke producten zoals melk. In dit onderzoek is gebleken dat in tegenstelling tot deze aanname, enkele PAKs wel overdragen naar melk, vooral de PAKs met minder dan vijf aromatische ringen. Daarnaast is gevonden dat een aantal PAKs overdragen in de vorm van metabolieten. Dit is van belang gezien het feit dat de carcinogene effecten van PAKs worden toegeschreven aan de metabolieten.
Verscheidene worst case scenario's zijn doorgerekend met gebruik van de maximum gehalten zoals gemeten in het Nationaal Plan voor diervoeders, en de (enkele) overdrachtspercentages die in de literatuur gevonden waren. Uit de resultaten bleek dat de inname van PAKs door koeien het niveau van milligrammen kan bereiken. De berekende inname door mensen via melk is tenminste een factor 1000 onder de toxicologische grenswaarden voor niet-carcinogene effecten. Daarentegen overschreed de berekende inname van carcinogene PAKs de 'virtually safe dose' voor Benzo(a)Pyreen. Hoewel de scenario's conservatief zijn vanwege het gebruik van maximum gehalten, zijn niet van alle categorieën diervoederingrediënten meetgegevens beschikbaar, of slechts in zeer beperkte mate. Deze onvolledige dataset levert weer een zekere onderschatting van de inname.
Met overdracht van PAKs als metabolieten is tot op heden nog geen rekening gehouden in Nationaal Plan's voor dierlijke producten. Gezien de verwachte carcinogene werking van PAK metabolieten, is hun mogelijke aanwezigheid in dierlijke producten een belangrijk aandachtspunt.
Aanbevelingen:

- *Het verzamelen van meer informatie over mogelijke overdracht van PAKs en hun metabolieten van diervoeder naar melk en andere dierlijke producten.*
- *Het meewegen van de toxiciteit van mogelijke overgedragen metabolieten in de risicobeoordeling*
- *Het opnemen van ingrediëntcategorieën die een groot aandeel beslaan van het totale diervoederpakket in de monitoring, ook als deze niet als risicocategorie worden aangemerkt.*

Vluchtige organische verbindingen (VOCs)
VOCs zijn eenvoudige koolwaterstoffen afkomstig uit ruwe olie en de daaruit afgeleide producten. In voedselproductie worden VOCs voornamelijk gebruikt als extractiemiddel voor winning van olie uit zaden, wat kan leiden tot residuen in diervoeder en humane voedselproducten. Er is erg weinig bekend over toxiciteit van deze stoffen via orale blootstelling. Een worst case scenario op basis van maximum

gemeten concentraties en 100% overdracht van diervoeder naar dierlijke producten leidt niet tot overschrijding van de (weinige) toxicologische grenswaarden voor orale blootstelling. Daarnaast is het de verwachting dat de concentraties in de ingrediënten zullen afnemen naarmate deze verder verwerkt worden tot mengvoeder. Ook hier zijn niet van alle ingrediëntcategorieën meetgegevens beschikbaar. Het is niet bekend in hoeverre (toxische) VOC metabolieten in landbouwhuisdieren worden gevormd en overgedragen naar dierlijke producten, daarom kon het mogelijke risico van inname door de consument niet worden uitgesloten. Uit de risicobeoordeling is verder gebleken dat nadelige gezondheidseffecten bij landbouwhuisdieren niet zijn uitgesloten.
Aanbevelingen:

- *Het meten van VOC concentraties in mengvoeder voor een nieuwe risicobeoordeling gericht op diergezondheid.*
- *Het verzamelen van informatie over de vorming en overdracht van toxische metabolieten van VOCs in diervoeder naar dierlijke producten*

<u>Algemeen</u>

- Uit de risicobeoordelingen van PAKs en VOCs kan geconcludeerd worden dat overdracht van metabolieten uit stoffen in diervoeder naar dierlijke producten aandacht behoeft. In het algemeen is de monitoring enkel gericht op de uitgangsstof.
- In de monitoring zou meer rekening gehouden moeten worden met de daadwerkelijke consumptie van diervoederingrediënten. Categorieën die normaal gesproken niet als 'risicomaterialen' worden beschouwd, kunnen met een lage concentratie contaminanten toch een aanzienlijke inname veroorzaken doordat ze een groot gedeelte van het totale diervoederpakket omvatten.
- Hulpstoffen zoals gedefinieerd in Verordening 1831/2003 mogen worden toegepast in de productie van diervoeder zolang de residuen geen gevaar vormen voor mens of dier. Zoals in het geval van de VOCs, kan het zijn dat er geen orale toxiciteitstudies beschikbaar zijn om nadelige effecten uit te kunnen sluiten. Dit zou er toe kunnen leiden dat zulke stoffen toch gebruikt worden in de voedselproductie. Het is daarom ten aanzien van hulpstoffen de vraag in hoeverre de Verordening effectief is in het beschermen van de gezondheid van mens en/of dier.

Contents

1 Introduction

Contaminants from feed materials may end up in edible animal products and as such may pose a possible risk to consumers. The level of contamination of the Dutch animal feed is monitored by means of the National Control Plan for 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. The latter compounds were nickel, mineral oils, poly aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs). To evaluate if these compounds should be included in the National Control Plan for Animal Feed in future years and/or maximum permissable concentrations should be established, initial risk assessments were performed. For this, toxicological profiles of the compounds were made, and data from the National Control Plan for Animal Feed were used for scenario calculations. For PAHs, additional information on PAH carry-over from the report 'Voorkomen van PAK's in voer, omgeving van dieren, melk en zuivelproducten alsmede een oriënterende studie in melkvee' of Kan et al (2003) was used. The risk assessments were performed in 2005 and 2006.

2 Nickel in mineral supplements

2.1 Introduction

Nickel is a very abundant natural element. Pure nickel is a hard, silvery-white metal. Nickel can be combined with other metals, such as iron, copper, chromium, and zinc, to form alloys. These alloys are used to make coins, jewelry, and items such as valves and heat exchangers. Most nickel is used to make stainless steel.
Nickel can combine with other elements such as chlorine, sulfur, and oxygen to form nickel compounds. Many nickel compounds dissolve fairly easy in water and have a green color. Nickel compounds are used for nickel plating, to colour ceramics, to produce certain types of batteries, and as catalysts that increase the rate of chemical reactions, e.g. to hydrogenate fats, which is the reason for the GMP-level in fat (Product Board Animal Feed, PDV).
Nickel is found in all soil and is emitted from volcanoes. Nickel is also found in meteorites and on the ocean floor. Nickel and its compounds have no characteristic odour or taste (ATSDR, 2003).

2.2 Occurrence

Nickel is an ubiquitous element in the soil at concentrations <1000 mg/kg, typical concentration range in soil is between 4 and 80 mg/kg. In the environment it is primarily found combined with oxygen (oxides) and sulphur (sulphides).
Especially under acidic conditions nickel is available for plant uptake via the roots from the soil. The nickel amount in most natural vegetation range from 0.05 to 5 mg/kg dry weight. At concentrations of 50 mg/kg (dry matter) nickel is expected to be toxic for plants. High concentration factors have been found in aquatic plants (bioconcentration factors of up to 20 000).
In wildlife, nickel is found in many organs and tissues, due to dietary uptake by herbivorous animals and their carnivorous predators. However, there is no evidence for the biomagnification of nickel in the food chain (ATSDR 2003, WHO working group on nickel, 1991).

2.3 Toxicological profile (hazard assessment)

2.3.1 General

By some authors nickel is considered an essential element for higher animals and humans, although its functional importance has not been demonstrated.
In terms of human health, toxic effects are mainly reported after inhalation of nickel. Via this route, nickel carbonyl is the most acutely toxic nickel compound, acute poisoning results in pulmonary symptoms are similar to those of a viral pneumonia. The liver, kidneys, adrenal glands, spleen, and brain are also affected. Chronic effects such as pulmonary and nasal changes and asthma have been reported in nickel refinery and nickel plating workers. Epidemiological studies have demonstrated a strong association between lung and nasal cancers and exposure by inhalation to nickel compounds in nickel refineries, primarily (in) the early stage of nickel refining. Because of this, nickel compounds are classified as carcinogenic to humans (Group 1), metallic nickel is classified as possibly carcinogenic to humans (Group 2B; IARC, 1997).

Cases of nickel poisoning have also been reported in patients dialysed with nickel-contaminated dialysate and in electroplaters who accidentally ingested water contaminated with nickel sulphate and nickel chloride.
Nickel and nickel compounds have a strong sensitising potential on the skin, which is manifested by irritation, eczema and allergic contact dermatitis. Oral intake of low doses of nickel may provoke allergic dermatitis in sensitised individuals (ATSDR, 2003).

2.3.2 Combination toxicology

Although nickel is known to interfere with iron metabolism, no other combination effects of nickel have been reported.

2.3.3 Absorption, distribution, metabolism and excretion (ADME)

2.3.3.1 Absorption

Studies with experimental animals and human volunteers have demonstrated a low absorption (less than 1%) from food (and feed), but oral absorption from drinking water is higher, reaching 25% (nickel sulphate was administered in this case; ATSDR, 2003). The gastrointestinal absorption is depending on chelating agents and pH. Iron also seems to affect the gastrointestinal absorption of nickel. It can be concluded that the presence of food limits the absorption of nickel and that more water-soluble nickel compounds are absorbed better than less soluble ones.

2.3.3.2 Distribution

Once taken up, nickel is bound to serum proteins. It is concentrated in kidney, liver, lungs and lymph nodes.

2.3.3.3 Metabolism and excretion

Elimination half life of absorbed nickel is about 28(± 9) hours (ATSDR, 2003).

2.4 Human oral exposure

Because of the scope of this risk assessment, the following paragraphs will focus on human oral exposure.

In 2005 the European Food Safety Authority (EFSA) estimated the human intake of nickel from the average diet to be about 150 µg/day (2.5 µg/kg body weight/day), but may reach 900 µg/day (15 µg/kg body weight/day) or more, when large amounts of food items with high nickel contents are consumed. Products with high nickel concentrations are cacao and chocolate, nuts, pod fruit (including soy beans and soy products), peanuts, liquorice and whole meal products (Voedingscentrum, 2005). In addition, first-run drinking water, which may contain up to 1000 µg/L, and leaching from kitchen utensils into food may also contribute to nickel intake. Intakes of 150 and 900 µg/day are about 500 and 90-fold lower, respectively, than the lowest dose reported to cause adverse effects in rats. Average intakes from food are about one third of the lowest intake reported to aggravate hand eczema in nickel sensitised subjects (EFSA, 2005).

2.5 Toxicological reference limits

National and international organisations reported tolerable daily intake limits (TDI), ranging from 5 to 50 µg/kg bw/day. EFSA was not able to derive a TDI in de absence of adequate data, but as little as 8 and 12 µg nickel/kg body weight could provoke hand eczema in nickel-sensitised individuals. See also table 1.

World Health Organisation (WHO, 2004): TDI 5 µg/kg bw/day
This TDI was derived from a NOAEL of 5 mg/kg of body weight per day from a dietary study with rats in which altered organ-to-body weight ratios were observed, using an uncertainty factor of 1000 (100 for inter- and intraspecies variation and an additional factor of 10 to compensate for the lack of adequate studies on long-term exposure and reproductive effects, a lack of data on carcinogenicity by the oral route and a much higher intestinal absorption when taken on an empty stomach in drinking-water than when taken together with food). The provisional drinking water quality guideline is 20 µg/L (by assuming a 60 kg adult drinking 2 litres of water and allocating 10% of the TDI to drinking water).

WHO (background document drinking-water quality, 2005): TDI 12 µg/kg bw/day
A critical NOAEL of 2.2 mg nickel/kg bw was based on the results of a two generation study with rats. An (standard) uncertainty factor of 100 was applied to the NOAEL. However, this may not be sufficiently protective for nickel sensitized individuals. The drinking-water guideline value is derived using the LOAEL of 12 µg/kg bw established after provocation of fasted patients with an empty stomach. This LOAEL is based on a highly sensitive part of the human population no safety factors have to be used a guideline value of 70 µg/L could be determined from this TDI by assuming a 60-kg adult drinking 2 litres of water and allocating a conservative 20% of the TDI to drinking-water.
The LOAEL is based on the total exposure to nickel, in this study, being from drinking-water, and the absorption of nickel from drinking water on an empty stomach is 10- to 40-fold higher than the absorption from food. Basing the total acceptable intake for oral challenge from studies using drinking-water on an empty stomach in fasted patients can, be considered a worst-case scenario.
Note: this revision of the limit has not been incorporated in the official WHO documents.

Agency for Toxic Substances and Disease Registry (ATSDR, 2003): no limits were derived.
Intermediate-duration studies suggest that the developing organism may be a sensitive target of nickel toxicity. Due to inadequate studies no acute- or intermediate-duration oral MRL has been derived. Also the data on chronic toxicity were considered to be inadequate to derive a chronic MRL.

European Food Safety Authority (EFSA, 2005): no limits were derived.
EFSA was not able to derive an upper level for the intake of nickel from food that is unlikely to pose a risk of adverse health effects. Perinatal mortality was reported to be increased in the offspring of female rats ingesting nickel salts, even at the lowest administered dose (1.3 mg nickel/kg body weight/day). Individuals sensitised to nickel through contact and who have allergic contact dermatitis develop effects at lower doses. It is not possible to derive a threshold for provoking dermal reactions in nickel-sensitised subjects. Although only dermal exposure to nickel can lead to sensitisation, oral doses of nickel have been shown to exacerbate hand eczema in nickel-sensitised individuals. In some studies, as little as 8 and 12 µg nickel/kg body weight provoked such reactions. In absence of adequate dose-response data for these effects EFSA considered it not possible to establish a TDI.

US-Environmental Protection Agency (EPA,1996): RfD 20 µg/kg bw/day for water-soluble nickel salts
This limit is based on decreased body and organ weights in a 2-year feeding study in rats. The NOAEL was 5 mg/kg bw/day in addition to the standard uncertainty factor of 100 an additional factor 3 was used to account for inadequacies in the reproductive studies. EPA has medium confidence in this RfD, based on high mortality in the control group.

National Institute for Public Health and the Environment (RIVM, 2001): TDI 50 µg/kg bw/day
This TDI is based on the same NOAEL as used by the WHO in 1996 and 2004. The RIVM established this TDI in an evaluation in 1991. An uncertainty factor of 100 was used.

Table 1: Overview of current toxicological safety limits for nickel.

			Study used							
Organi-Sation	Limit type	Limit value	Exposure	Route	Species	Critical dose	Endpoint	Parameter	Reference	Uncertainty factor
WHO (2004)	TDI	5 µg/kg bw	2 year	Oral food	Rat	5 mg/kg bw/day	NOAEL	Decreased organ and body weight	Ambrose et al. 1976	1000
WHO (2005)	TDI	12 µg/kg bw	Single dose	Oral drink	Fasted human	12 µg/kg bw	LOAEL	Eczematous reaction	Nielsen et al. 1990	0
ATSDR	-									
EFSA	-									
EPA (1996)	RfD	20 µg/kg bw Soluble Ni salts	2 year	Oral food	Rat	5 mg/kg bw/day	NOAEL	Decreased organ and body weight	Ambrose et al. 1976	300
RIVM (2001)	TDI	50 µg/kg bw	2 year	Oral food	Rat	5 mg/kg bw/day	NOAEL	Decreased organ and body weight	Ambrose et al. 1976	100

2.6 Commodity limits

No regulatory limits have been established for feed materials; nickel is not included in the list of undesirable substances in animal feed (directive 2003/32 and amendments). No regulatory limits have been established for products (food) of animal origin. In the Netherlands as part of the GMP regulations an action level of 20 mg/kg (in fat) and rejection level of 50 mg/kg has been established (GMP 14; 17-08-2005).

2.7 Measurements in feed materials

Analyses performed by RIKILT in 2001 – 2004 revealed that concentrations range between 2 and 647 mg/kg (table 2). These concentrations are higher than those analysed by PDV in feed ingredients. They found maximum concentrations, in vegetable oil, ranging between 5 and 10 mg/kg (data not shown).

Table 2: Nickel concentrations (in mg/kg) in mineral mix and premix for animal feed. Data from 2001-2004. dl = detection limit of 0,01 mg/kg. Type of animal feed from which the maximum concentration originated is specified in the last column.

Year	Maximum	Median	# samples	# > dl	% > dl	Specification
2001	45.5	0.28	26	6	23	Additional cattle feed
2002	518	93	19	19	100	Mineral mix cattle
2003	647	26.05	48	48	100	Premix
2004	228	21	44	41	93	Mineral mix additional cattle feed

2.8 Transfer from animal feed to products for human consumption

From the reviewed literature, no data could be found concerning the transfer of nickel from feed to either meat or milk.

2.9 Considerations for risk assessment

- Human intake of nickel is mainly originating from consumption of vegetable products, the contribution from animal products is marginal.
- It is unclear if nickel is transferred from animal feed to edible animal tissues such as meat, or milk.
- It is unclear to what extent mineral supplements (with its possible nickel content) are used in relation to the total amount of animal feed.
- The human daily intake as estimated by EFSA is at the level of the lowest TDI. It is questionable if occasional high concentrations of nickel in animal feed will add significantly to this intake.

2.10 Conclusion

The additional intake of nickel originating from mineral feed mixes to the human daily intake is considered to be marginal. An extended risk assessment using data from the National Control Plan for Animal Feed was therefore not performed.

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3 Mineral oils

3.1 Introduction

Food grade mineral oils, or hydrocarbons, are produced as by-products during the refining and distillation of crude mineral oils. They may be obtained from crude oil sources of naphthenic (N) or of paraffinic (P) origin and by either the conventional acid (oleum)-treatment process (A) or the hydrogenation or hydrotreatment process (H).
Mineral hydrocarbons have been widely used in many different applications which may potentially give rise to residues in food. Past or present uses include direct food additives (e.g. as glazing agents), constituents of chewing gum base, processing aids (e.g. lubricants), in food packaging materials (e.g. wax-coated cartons), in cheese wax, in pesticide formulations, as adjuvant in veterinary medicines, and in human medicines (e.g. liquid paraffin laxative). Within Europe, their use as direct food additive has diminished and in some countries they are not permitted for food additive use any longer (SCF 1995).

3.1.1 Definition

The characteristics of mineral oils in relation to molecular weights, viscosity and chemical structure are poorly defined. The analytical definition of mineral oils is: 'the sum of all structures between the boiling points of n-C10 and n-C40, which are extractable with an apolar solvent and detectable with gas chromatography' (NEN 5733). In short: all straight alkanes with carbon numbers between 10 and 40. White mineral oils are highly refined mineral oils which are composed of saturated iso-alkane (paraffinic) or cyclo-alkane (naphtenic) hydrocarbons. These oils are essentially free of aromatics, unsaturated compounds, and impurities such as sulphur, nitrogen, oxygen, halides and metals. Mineral oil is also referred to as (mineral) hydrocarbons.

Their viscosity ranges from 10-100 centistokes (cSt1, 10-100 mm2/s). Thus a P100(H) oil refers to a paraffinic oil with a viscosity of 100 cSt produced by the hydrogenation process and a N10(A) oil to a naphthenic oil with a viscosity of 10 cSt produced by the acid-treatment process (FAO/WHO, 1995). There are three main types of products; liquids, semi-liquids and solids, examples of each being mineral (white) oils, petroleum jellies and mineral waxes (SCF, 1995). They can be approximately classified as follows (EMEA, 1995):
Low and medium viscosity: C10-C25, viscosity at 100 degrees: 3-8.5 cSt, molecular weights 300-500
Microcrystalline waxes: C20-C60, viscosity at 100 degrees: 10-30 cSt, molecular weight 300-750+
High viscosity: >C30, viscosity at 100 degrees: > 11 cSt, molecular weight >500.

This document focuses on the effects of the aliphatic alkanes with chain lengths between C10 and C40 which are present in mineral oil, since it was this fraction which was analyzed in animal feed until 2005 by RIKILT Institute of Food Safety for the National Control Plan for Animal Feed. Where relevant, effects of other compounds which may be present in mineral oil are discussed.

1 Viscosity is a measure of the resistance of a fluid to deform under shear stress. Kinematic viscosity (Greek symbol: ν) has SI units ($m^2 \cdot s^{-1}$). 1 centistokes = 1 mm^2/s

3.2 Occurrence

Mineral oil may occur in feed and food as a result from contamination with petroleum products, or leaching from package materials into the products. Grob et al. (2001) have conducted a study in 1999 on the presence of mineral oil in food and feed stuffs. In animal body fat, they found an average concentration of 25 mg/kg in the fat phase, with a maximum of 150 mg/kg. In eggs, an average concentration of 3 mg/kg with a maximum of 80 mg/kg was found.
Mineral hydrocarbons, which were supposed to originate from food, have been found in human breast milk (Noti et al., 2003). The chain lengths were between C15 and C45, the average molecular weight was between C23 and C33, and often more than half were below C25. Concentrations ranged from 25 to 200 mg/kg fat and were considered as a background concentration common to all women.
In addition to hydrocarbons originating from mineral oil, the diet will contain other hydrocarbons due to biosynthesis of hydrocarbons in terrestrial and marine plants and animal species. Saturated and unsaturated n-alkanes with chain lengths of C15, Cl7 and C21 are predominant in marine organisms, whilst saturated n-alkanes of chain lengths C27, C29 and C31 are typical of terrestrial plants.

3.3 Toxicological profile (hazard assessment)

3.3.1 General

SCF reviewed unpublished reports in 1995 and found the following effects after oral exposure to mineral oils (of which the exact composition is not clear): increased organ weights, especially liver and lymph nodes; altered serum enzyme levels; increased monocyte and neutrophil counts; reduced red blood cells, haemoglobin, haematocrit, MCHC, MCH; and the accumulation of hydrocarbon material in tissues. The main histopathological findings were granulomata in the liver and focal collections of vacuolated macrophages (histiocytosis) in the lymph nodes. In animals dosed with certain waxes, an inflammatory lesion at the base of the mitral valve in the heart was observed.

Studies in rats and dogs have revealed that ingestion of highly refined white mineral oils induced effects on liver and mesenteric lymph nodes, as earlier found by SCF. Effects included increased organ weight, microscopic inflammatory changes, and the accumulation of saturated mineral hydrocarbons in affected tissues and occurred after intake of a dietary dose of 20 ppm for 90 days. Effects occurred only in F344 rats, no statistically significant effects were found in Beagle dogs and Sprague-Dawley rats (Smith et al., 1995 and 1996). In addition, Scotter et al. (2003) found comparable effects in F344 rats after 28-day and 90-day oral exposure to 2% of several types of mineral oils in the diet. They identified from chemical analysis from affected tissues, that it was most likely the fraction of straight and branched alkanes between C20-C35 that was responsible for the observed effects.

SCF concluded earlier (1995): ‘It is not possible to predict the ultimate consequences for health of the reactions observed in some of the animal studies, such as the inflammatory reactions in the liver and the mitral valve of the heart. However, we consider that these and some of the other effects noted above are undesirable and further consider that there are sufficient parallels between the observations of accumulation and effects in animals and man to conclude that there is the potential, depending on the intake, for adverse effects on human health.’
In addition, they concluded that it is largely the amounts of lower molecular weight, shorter chain-length substances, which are absorbed and only slowly cleared from the body, that most probably

determine the occurrence or absence of toxicity, rather than the presence of very small proportions of unusual, highly toxic components.

Hepatic lipid granulomas have been seen in humans exposed to mineral oils through the diet and by ingestion of medicinal mineral oils. Doses associated with the effect in humans are not known (ATSDR, 1999). A group of pathologists concluded that these granulomas were not the same as those observed in the F344 rats and were inconsequential in humans (Carlton et al., 2001).

3.3.2 Combination toxicology

From the reviewed literature, no combination effects of the separate compounds in mineral oil could be identified. Since mineral oils are complex mixtures of alkanes and other compounds, it is possible that interactive effects between the separate ingredients occur. Since mineral oils are usually tested as the whole mixture, it is not possible to elucidate the occurrence and/or nature of possible combination effects.

3.3.3 Absorption, distribution, metabolism and excretion (ADME)

ADME processes are considered and described for the separate alkane fractions and taken mainly from the ATSDR toxicological profile on Total Petroleum Hydrocarbons (1999). This profile builds on the efforts by the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG, 1997a and b) and Massachusetts Department of Environmental Protection (MADEP) to group chemicals into fractions with similar environmental transport characteristics (i.e., transport fractions). Fractions are identified by dividing aromatic and aliphatic hydrocarbons by structure and further subdividing on the basis of equivalent carbon number 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. Only aliphatic alkane fractions are described hereafter.

3.3.3.1 Absorption

EC>8-EC16 and EC>16–EC35 fractions: no studies were found regarding absorption of hydrocarbons in these fractions after oral exposure in humans. Studies in rats show that absorption of ingested aliphatic hydrocarbons is inversely related to molecular weight, ranging from complete absorption at the lower end of the molecular weight range to about 60% for C14 hydrocarbons, 5% for C28 hydrocarbons, and essentially no absorption for aliphatic hydrocarbons with >32 carbons (ATSDR, 1999). It is commonly assumed that n-alkanes of carbon numbers >29 are not significantly absorbed from the gastrointestinal tract (EMEA, 1995).

3.3.3.2 Distribution

EC>8 –EC16 fraction: aspiration to the lungs may occur following ingestion of hydrocarbons in this fraction (Cavender, 1994, cited by ATSDR, 1999). Following absorption from the gastrointestinal tract, smaller molecular weight aliphatic hydrocarbons and/or their metabolites are transported in the body via the blood and the lymph system, whereas larger molecular weight aliphatic hydrocarbons may be distributed predominately via the lymph system (Review Albro and Fishbein, 1970; Miller et al., 1996 cited by ATSDR, 1999).

EC>16 –EC35 fraction: following absorption, hydrocarbons in this fraction may be expected to accumulate to some degree in liver and fatty tissues, as indicated by the observation that, 24 hours after administration of an oral dose of tritiated mineral oil to rats, concentrations of tritiated mineral oil were about 7-fold greater in fatty tissues and liver than in kidney and brain (ATSDR, 1999). Lipogranulomata (clusters of lipoid droplets surrounded by lymphocytes and macrophages) are

commonly found in human autopsies, particularly in liver, spleen, and abdominal lymph nodes. These structures are associated with dietary exposure to mineral oils and waxes, and are considered a benign response without adverse consequences (Miller et al., 1996; Wanless and Geddie, 1985, cited by ATSDR, 1999).

3.3.3.3 Metabolism

EC>8 –EC16 fraction: hydrocarbons in this fraction are oxidatively metabolized to fatty acids and alcohols, apparently mediated by cytochrome P-450 isozymes. Studies regarding the metabolism of hydrocarbons in this fraction in humans or animals provide suggestive evidence that metabolism may be slow. Studies in rats indicated that aliphatic hydrocarbons in this fraction may be metabolized more slowly than aromatic hydrocarbons of equivalent molecular weight (Zahlsen et al., 1992 and Miller et al., 1996, cited by ATSDR, 1999).
EC>16 –EC35 fraction: aliphatic hydrocarbons in this fraction are not expected to undergo extensive metabolism in animals or humans. In monkeys, 2 days after intramuscular injection of a mineral oil emulsion with a radiolabeled C16 hydrocarbon (n-hexanedecane), substantial portions (30-90%) of radioactivity in various tissues existed as unmetabolized n-hexanedecane. The remainder of the radioactivity was found as phospholipids, free fatty acids, triglycerides, and sterol esters. No radioactivity was found in water-soluble fractions (ATSDR, 1999).

3.3.3.4 Excretion

EC>8 –EC16 fraction: results from studies with humans exposed by inhalation to white spirit (a mixture of C10-C12 aliphatic hydrocarbons) suggest that hydrocarbons in this fraction are slowly eliminated following distribution to fatty tissues (Pedersen et al., 1984, cited by ATSDR, 1999). No studies were located regarding the routes of excretion for this fraction of hydrocarbons in humans or animals.
EC>16 –EC35 fraction: hydrocarbons in this fraction may be expected to be eliminated predominately in the faeces, based on experiments with rats given oral or intraperitoneal doses of tritiated mineral oil. With oral exposure, 90% of administered radioactivity appeared rapidly (within 2 days) in the faeces, predominately as unchanged mineral oil; less than 10% of administered radioactivity appeared in the urine within 2 days of administration. (ATSDR, 1999).

3.4 Human oral exposure

No data regarding the dietary intake of the Dutch population could be found. A study in the USA estimated a total dietary intake of mineral hydrocarbons of 0.875 mg/kg bw/day, of which 0.427 mg/kg bw/day is of white mineral hydrocarbons (Heimbach et al., 2002). This corresponds with findings in the UK, where an average daily intake of 0.47 mg/kg bw/day was calculated for adults (Food Chemical Risk Analysis 2001, cited by Noti et al., 2003). For preschool children the corresponding value was 0.98 mg/kg bw/day. The Committee on Toxicity of Chemicals in food (COT, 2003) have estimated an average daily intake of 0.024 mg/kg bw/day of mineral oils originating from package materials in the UK. The separate fractions in this last study could not be identified, but hydrocarbons with chain length between C16 and C35 were analysed.

3.5 Toxicological reference limits

Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995/2002): ADI 0-20 mg/kg bw (high viscosity mineral oil: boiling point above 350 ˚C).
Revised ADI (2002, low- and medium viscosity mineral oil: boiling point above 200 ˚C)

- 0-10 mg/kg bw for class I: viscosity 8.5-11 mm2/s, carbon number at 5% distillation point not less than 25, average molecular weight 480-500, including P70(H) oil;
- 0-0.01 mg/kg bw (group ADI, temporary) for class II: viscosity 7.0-8.5 mm2/s, carbon number at 5% distillation point not less than 22, average molecular weight 400-480, including N70(H) and N70(A) oils;
- 0-0.01 mg/kg bw (group ADI, temporary) for class III: viscosity 3.0-7.0 mm2/s, carbon number at 5% distillation point not less than 17, average molecular weight 300-400, including P15(H), N15(H), and N10(A) oils.

Temporary ADIs will be re-evaluated by JECFA in 2006.

TPHCWG (1997), ATSDR (1999), US-EPA (2004) and RIVM (1999) adopted identical limits, summarized in table 1. In general, the organisations have used a comparable approach in setting toxicological safety limits for total petroleum hydrocarbons (TPH), under which are the mineral oils. The methodology selected was termed the indicator/surrogate approach. The indicators referred to are the single compounds in petroleum which are known to be carcinogens and which are evaluated/regulated individually. If indicator compounds are not present or are below regulatory criteria, such as in food-grade mineral oils, hazard assessment for TPH fractions is utilized. For this, separate fractions are determined on the basis of structure and equivalent carbon number index (EC, see paragraph 3.3.3). A surrogate (single compounds or, preferably, mixtures) is selected which best represents the composition of each fraction. The RfDs of the whole fraction have been developed by extrapolation of all available toxicity data of the surrogates. Differences in approach by the organisations are specified below.

Table 1: Safety limits for separate alkane fractions of total petroleum hydrocarbons, as adopted by TPHCWG, ATSDR, US-EPA and RIVM. US EPA has modified the RfD for the fraction EC8-EC16 in 2004.

Aliphatic Total Petroleum Hydrocarbon fraction	Oral RfD/TDI mg/kg bw/day	Uncertainty factor applied
>EC8-EC16	0.1 (US EPA, 2004: 0.03)	1000/5000
>EC16-EC35	2.0	100
>EC35	20	100

TPHCWG (1997): surrogate approach.
The TPHCWG approach assumes additivity of the surrogate compounds and the hydrocarbon fractions in assessing the potential for adverse effects of TPH on health. RfDs are set using the following surrogates:
EC>8-EC16 fraction: using RfD and RfC for dearomatized petroleum streams (white spirit).
Toxicity data on individual components in the aliphatic >EC8-EC16 fraction are minimal.
The data used were from studies on jet fuel JP-8 (EC9-EC16) and on de-aromatised

petroleum streams (10 studies in total), which, together, cover the entire range of the fraction. Although data on n-nonane are available, the data on petroleum streams were preferred since these data refer more to mixtures rather than on an individual compound at the low end of the fraction. The uncertainty factors (UF) used in these studies varied from 1000, which includes an additional factor of 10 to compensate for the use of subchronic studies, to 5000 in one study. The additional factor of 5 was applied to compensate for the use of a LOAEL instead of a NOAEL.
EC>16-EC35 fraction: using RfD for white mineral oils.
The RfDs for the aliphatic >EC16-EC35 and >EC35 fractions were based on an extensive British Industrial Biological Research Association (BIBRA) in Fischer 344 (F/344) rats as reported by Smith et al., (1996) in which Fischer rats were exposed to various white mineral oils. The RfD is based on the NOAEL for low molecular weight oils for liver granulomas (200 mg/kg bw per day) and a UF of 100.
EC>35 fraction: the RfD for the >EC35 fraction (20 mg/kg bw per day) is based on the NOAEL for high molecular weight oils for liver granulomas (2000 mg/kg bw per day) and a UF of 100.

ATSDR (1999): adapted surrogate approach of TPHCWG.
A notable difference between ATSDR and the other groups is that the other groups have focused on longer-term exposure scenarios, whereas ATSDR is concerned with the entire spectrum of possible exposure periods from acute to chronic. Surrogates were:
EC>8-EC16 fraction: using a chronic inhalation MRL for JP-7.
EC>16-EC>35 fraction: using health effects data for mineral oils, but no MRLs are available

US-EPA (2004): adapted surrogate approach TPHCWG.
US-EPA adopted the limits as set by the TPHCWG in 1997, but applied several changes in 2004, under which for the following aliphatic fraction:
EC>8-EC16 fraction: the oral reference dose (RfD) for this fraction was based on TPHCWGs analysis, plus an additional safety factor of 3 as recommended by US-EPA.

RIVM (1999): identical to TPHCWG.
All human toxicological TDIs for relevant TPH fractions are based on the criteria set by the TPHCWG.

SCF (1995): several group ADIs.
SCF allocated a full group ADI of 0-20 mg/kg bw for waxes conforming to the following specification:
- Highly refined waxes derived from petroleum based or synthetic hydrocarbon feed stocks, with Viscosity not less than 11 cSt at 100°C;
- Carbon number not less than 25 at the 5% boiling point;
- Average molecular weight not less than 500;

Any further data on these waxes was not required.

SCF allocated a temporary group ADI of 0-4 mg/kg bw for oils conforming to the following specification:
- White paraffinic mineral oils derived from petroleum based hydrocarbon feed stocks, with Viscosity not less than 8.5 cSt at 100°C;
- Carbon number not less than 25 at the 5% boiling point;

- Average molecular weight not less than 480.

A chronic toxicity/carcinogenicity study of 2 years duration on P70(H) oil was required, which includes a reversibility phase in which a satellite group dosed for 1 year is left untreated for a further year, to be submitted within 4 years.

3.6 Commodity limits

PDV (2001): There are no EU- or national commodity limits for mineral oil in animal feed. PDV recommends action- and rejection limits for crude oils and fats in the animal feed sector (see table 2).

Table 2: Action/rejection limits for mineral oil in animal feed as proposed by PDV (2001)

Group compounds	Action/rejection limit (%m/m)
Animal fat (excl. fish oil)	0.04 (400 mg/kg)
Vegetable oil (excl. sunflower oil)	0.04 (400 mg/kg)
Vegetable fatty acids (incl. mixtures of fatty acids)	0.3 (3000 mg/kg)
Crude fish oil	0.3 (3000 mg/kg)
Sunflower oil and fatty acids	0.1 (1000 mg/kg)

3.7 Measurements in feed materials

As Grob et al. (2001) stated: it must be kept in mind that results on hydrocarbons do not refer to the total of the contamination. Furthermore, this concentration cannot be interpreted as an indicator of toxicity. The hydrocarbons are probably the least toxic waste components. There is no hint whether they are accompanied by PCBs or other highly toxic components, nor does a high concentration of mineral oil material necessarily go along with a high concentration of toxic material.

Analyses of mineral oil in animal feed have been performed by RIKILT from 2000 until 2005. The highest concentrations were found in 2000 in linol fat, with a concentration of 1.02% (m/m). The following years, concentrations were not higher than 0.14%, mostly in palm oil fatty acids. This last value is below the proposed rejection limit of 0.3 of PDV. See also table 3.

Table 3: Mineral oil concentrations in % (m/m) in fats and oils for use in animal feed. Data from 2000-2004. dl = detection limit of 0,04%. Fat type from which the maximum concentration originated is specified in the last column.

Year	Maximum	Median	# samples	# samples >dl	Specification fat
2000	1.02	0.18	59	17 (28%)	Linol fat
2001	0.14	0	97	4 (4,1%)	Bird fat
2002	0.05	0.05	50	1 (0,5%)	Palm oil fatty acid
2003	0.1	0.1	19	3 (16%)	Palm oil fatty acid
2004	0.14	0.1	16	3 (19%)	Palm oil fatty acid

3.8 Transfer from animal feed to products for human consumption

Although hydrocarbons have been found in goats milk (Cerbulis et al., 1985), it is unclear to what extent petroleum hydrocarbons are transferred from feed to milk or meat. Uptake of mineral oil by cows from feed can be concluded from the following studies by Coppock et al. (2001, 2002), who found temporary accumulation of n-alkanes (c10-c19) in the adipose tissue of cows exposed to crude oil and diesel in the feed. For the hydrocarbons with higher chain length (>C30) it is known that they, like in humans, are not absorbed in ruminants and have a laxative effect. Paraffin is also medicinally used for this purpose. Crude oil has been seen as having this effect in cattle (Poppenga, 2000).

Khan et al. (2005) have identified that oral exposure of cows to the crude oil and diesel as used in the studies by Coppock et al. (2001, 2002) resulted in a decrease in biochemical reactions of polymorphonuclear leukocytes (phagocytosis pathway), thus affecting immunity of the ruminants, even at concentrations as low as 0.005% (v/m) crude oil and 0.0025% (v/m) diesel. Earlier, they had discovered induction of P450 enzymes in rats after oral exposure to the same crude oil as used in the study in cattle. In this study, they also had found similar accumulation of n-alkanes (C10-C19) in the adipose tissue from the rats (Khan et al., 2002). It is not clear which substances in the dosed oils are responsible for the effects seen in rats and cattle, induction of the P450 enzymes indicates that it might be Ah-receptor mediated, suggesting that compounds like PAHs could be responsible. However, it has been shown that alkanes from oil and diesel are taken up and distributed in the cow, and could thus be present in milk and meat meant for human consumption.

3.8.1 Risk for animal health

Assuming that approximately 1.4% fat is used in the production of feed for cows (Kemme and Van Raamsdonk, 2004), combined with the measured concentration of 0.14 % in palm oil fatty acids, a concentration of 0.002% could be present in cow feed. This value of isolated alkanes is similar to the 0.0025% diesel concentration in animal feed which induced immunotoxic effects in cattle. It may be expected that the total concentration of the product from which the measured alkanes originated is higher.

Another matter of interest is that the concentrations which induce these effects on immune response in cattle are below those proposed by PDV.

3.9 Considerations for risk assessment

Alkanes or hydrocarbons below C30 are taken up by mammals and subsequently induce effects. In rats, effects are microgranulomas in liver and mesenteric lymph nodes, but only in F344 rats and not in other rats or in dogs. In humans, comparable accumulations of alkanes in liver and lymph nodes after ingestion of mineral oil have been found.

The relevance of these effects for humans is not clear. There is no information on the extrapolation from this specific strain of rats to humans. SCF concluded that these effects are undesired and are related to the alkanes rather than occasional very toxic compounds in the mineral oil. The opinon of a panel of pathologists that the observed effects in rats and humans are of non-adverse nature has not led to a review of SCF's opinion. Finally, several organisations have derived safety limits based on the effects in F344 rats.

A different effect seen in animals after oral exposure to mineral hydrocarbons is the suppression of immune response. In cows, biochemical reactions in the phagocytosis pathway of white blood cells were suppressed after oral exposure to crude oil and diesel. Although accumulations of alkanes were found in the fat tissue, it is not known which compounds in the oil were responsible for the effects. Exposure of rats to the same crude oil resulted in induction of P450 enzymes, indicating that activation of the Ah-receptor pathway may be involved. The presence of alkanes in the animal fat may indicate that they could also be transferred to milk, thus additionally exposing humans.

In humans, mineral hydrocarbons were detected in breast milk. The hydrocarbons were supposed to originate from food, and thus absorbed by humans. Mineral oils in the human diet can originate from contamination of animal feed or food (oils or lubricants which can leak from machinery into the feed/food during production) or application as food additive. Another route of exposure is from package materials, from which the hydrocarbons migrate into the food.

The amount of mineral oil in feed and food is usually measured by GC analysis of the C10-C40 hydrocarbon fraction in materials. Natural alkanes cannot be distinguished in this analysis (personal comment Wim Traag, RIKILT Institute of Food Safety). Additional to the effects induced by alkanes, it is expected that the overall toxicity of mineral oil is more dependent on the presence of other compounds, such as toluene, PCBs or PAHs. It has not been possible to correlate the results of alkane analysis to the presence of these latter compounds. The toxicological relevance of the hydrocarbon analyses or its indicator function for total contamination remains thus unclear. Considering all this, a full risk assessment using data from the National Control Plan for Animal Feed was not performed.

3.10 Conclusions and recommendations

- Oral exposure to alkanes induces microgranuloma in F344 rats, not in other rats or dogs. Similar phenomenen have been observed in humans after oral exposure to mineral oil. There is no consensus to whether the effects in rats and humans are of an adverse nature.

- The feasability of the TDIs for mineral hydrocarbons is questionable. Human total hydrocarbon intake from food-use applications exclusively already exceeds the low TDI for fraction EC8-16. The contribution of alkanes from this fraction to the total hydrocarbon intake is not clear. Analysis of hydrocarbons present in food or feed is usually not specified for the separate fractions, which is severely hampering the correlation of the exposure to the TDI. Detailed analysis of hydrocarbons (specified into separate fractions) in food and feed is recommended.

- There is very limited information on the transfer of separate fractions of hydrocarbons from feed to animal products. For a more detailed risk assessment, further research on this carry-over is needed.

- Alkane analysis can only be used as an indicator for incidents with mineral oil in animal feed, and not as indicator for the presence of highly toxic compounds.

- Alkane analysis can only provide a total alkane concentration. Natural alkanes cannot be distinguished from alkanes originating from mineral oil.

- Oral exposure of crude mineral oil in feed induces effects on the biochemical immune response in cows. Extrapolating results from alkane analysis in feed ingredients, the concentration in animal feed could be similar to the effect concentration. Proposed PDV levels exceed the effect concentration. The protection of animal health could be a factor in the decision on monitoring of mineral oil in animal feed.

- Considering the above mentioned uncertainties in interpreting the analytical results, inclusion of mineral oils in the National Control Plan for Animal Feed is not recommended.

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4 Poly Aromatic Hydrocarbons

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4.1 Introduction

In 2003 it was recognized that data on the carry-over of Poly Aromatic Hydrocarbons (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 National Control Plan for Animal Feed was not performed in this study.
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.

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.

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

Definition

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

United States Environmental Protection Agency US-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. PAHs in food may originate from air, soil or water by environmental contamination, or by PAH formation during processing and cooking. This 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).

4.2 Occurrence

Kan et al. (2003) reviewed the occurrence of PAHs in animal products. In France, PAHs have been found in milk at total concentrations of 37 and 27 ng/g fat (Grova et al., 2000 and 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).

4.3 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, development, and immunity (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 animal products.

4.4 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).

4.5 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

* 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 concentrations in food commodities (http://www.efsa.eu.int/science/data_collection/pah/1168_en.html)

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).

4.6 Toxicological reference limits

Based on existing NOAELs for non-carcinogenic effects of PAHs, the US EPA (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 National Control Plan for 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): drinking water guideline 0.07 µg/litre.
The 1993 Guidelines (retained in 1998) concluded that there were insufficient data available to derive drinking-water guidelines for PAHs other than BaP. The guideline value for BaP was based on a study in CFW mice which were fed BaP in the diet. In the mice 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): no reference limits derived.
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, 2002): virtually safe dose 0.14 ng BaP/kg bw/day.
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 (Integrated Risk Information System IRIS, 2002) an oral "virtually safe dose" for benzo[a]pyrene of 0.14 ng /kg bw/day was calculated for a cancer risk level of $1x10^{-6}$ via linear extrapolation.

Scientific Committee on Food (SCF, 2002): no reference limits derived.
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, 2001): VSD 0.5 ng PAHs /kg bw/day.
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 (1993, 1994): several RfDs.
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 (2001): TDI of 40 µg/kg bw/d for phenanthrene.
RIVM 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).

4.7 Human oral exposure

In a study of De Vos et al. (1990, cited by SCF, 2002) a worst case scenario was used for calculation, 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.

4.8 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 0.6-18.9 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. 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 National Control Plan for Animal Feed, the data will thus be expressed in weight only.

Data from the National Control Plan for 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 ANNEX I.

Table 2: Maximum concentrations of PAHs in feed ingredient categories as measured in the National Control Plan for 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.

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

4.9 Commodity limits

There are only few limits for 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).

4.10 Transfer from animal feed 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.

4.11 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 may 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 National Control Plan for Animal Feed on PAH concentrations in animal feed was used. On some matrices no or very limited data was available from the National Control Plan, for instance for fresh grass. On this last matrix, a brief literature review was undertaken resulting in data from a few studies.

4.11.1 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 Voedselveilgheid, 2005). For details on the method of calculation, see ANNEX I.

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 ANNEX I.

4.11.2 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 concentrations 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.

4.11.3 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 National Control Plan for Animal Feed. 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 for risk assessment results 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 concentrations lower than detection limit were assumed to be at the level of detection.

4.11.4 Scenario study ASG

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

4.12 Results

Data on intake and PAH concentrations were used to calculate the exposure of the cows and subsequently the expected concentrations in milk, based on carry-over rates. These concentrations were used to estimate the exposure of the consumer, based on a milk consumption of 1.5 liters 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.

4.12.1 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.

4.12.2 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.

4.12.3 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.

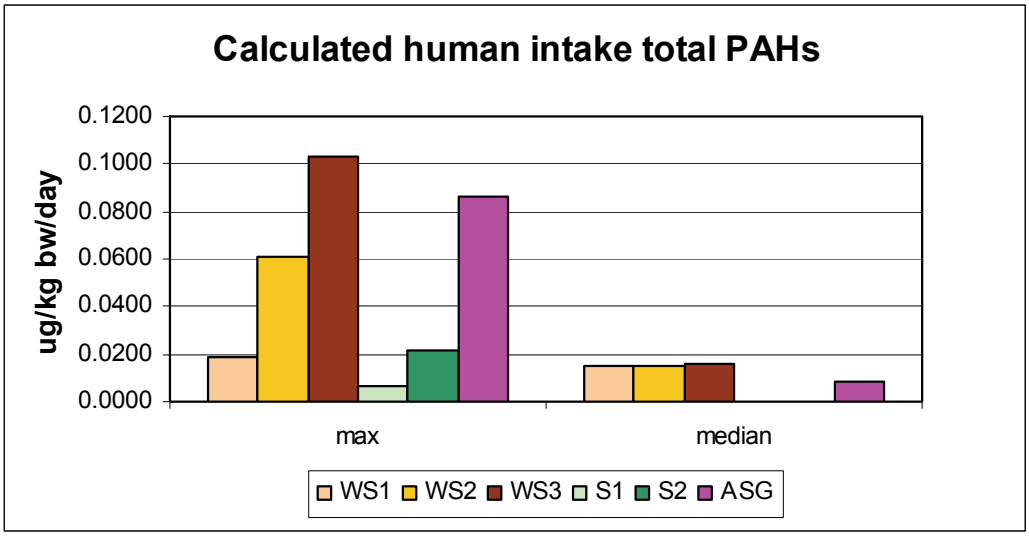

Figure 1: Calculated total human intake of PAH, based on maximum and median concentrations of PAHs in animal feed ingredients. Intake in µg/kg bw/day.

4.12.4 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 PAHs of 0.5 ng/kg bw/day (5 ng/kg bw/day for only BaP) is not exceeded.

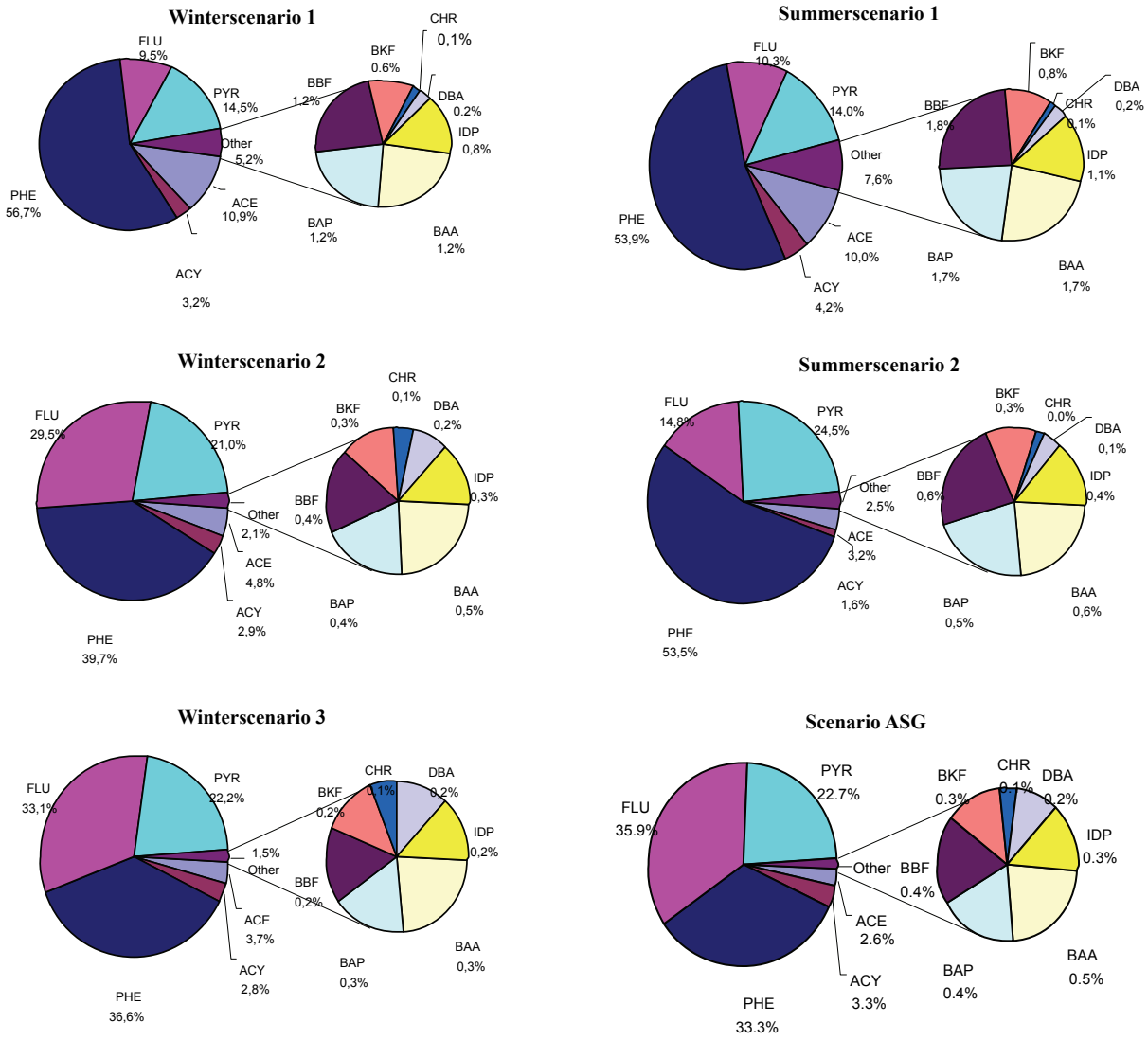

Figure 2: Contribution of individual PAHs to the calculated concentrations in milk for the worst case scenarios. Distribution in percentages. ACE=Acenaphthene, ACY=Acenaphthylene, BAA=Benz[a]anthracene, BAP=Benzo[a]pyrene, BBF=Benzo[b]fluoranthene, BKF=Benzo[k]fluoranthene, CHR=Chrysene, DBA=Dibenzo[a,h]anthracene,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

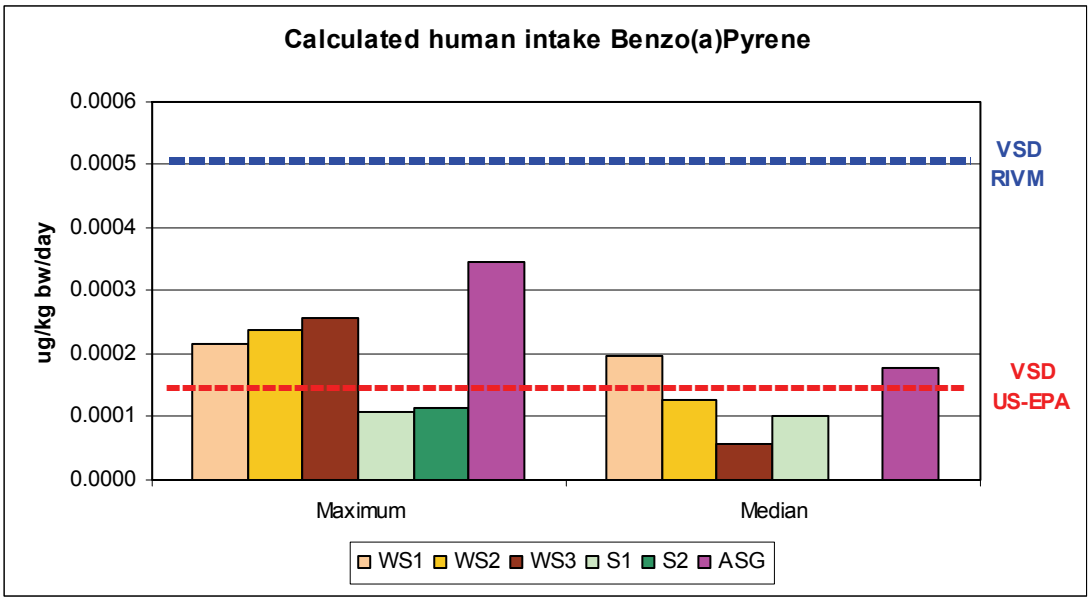

Figure 3: Calculated total human intake of Benzo(a)Pyrene, based on maximum and median concentrations of BaP in animal feed ingredients. Intake in µg/kg bw/day. For comparison, the 'virtually safe dose' for Benzo(a)Pyrene is also presented.

*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 are in µg/kg bw/day. Data on calculation and all PAHs can be found in ANNEXES I-VII. 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 $1*10^{-6}$.*

PAH:	**ACE**	**PHE**	**FLU**	**PYR**	**BAP**
RfD:	**60**	**40**	**40**	**30**	**0.00014***
Worst case scenario winter 1					
Intake human	0.002	0.0106	0.0018	0.0027	0.0002
Ratio RfD/intake human	30000	3774	22222	11111	0.70
Worst case scenario winter 2					
Intake human	0.0029	0.0242	0.018	0.0128	0.0002
Ratio RfD/intake human	20690	1653	2222	2344	0.70
Worst case scenario winter 3					
Intake human	0.0038	0.0378	0.0342	0.0229	0.0003
Ratio RfD/intake human	15789	1058	1170	1310	0.47
Worst case scenario summer 1					
Intake human	0.0006	0.0035	0.0007	0.0009	0.0001
Ratio RfD/intake human	100000	11429	57143	33333	1.40
Worst case scenario summer 2					
Intake human	0.0007	0.0116	0.0032	0.0053	0.0001
Ratio RfD/intake human	85714	3448	12500	5660	1.40
Scenario ASG					
Intake human	0.0025	0.032	0.0345	0.0218	0.0004
Ratio RfD/intake human	24000	1250	1159	1376	0.35

4.13 Discussion

The Dutch Food and Consumer Product Safety Authority (VWA) has the incentive to include certain contaminants in the yearly National Control Plan for Animal Feed based on risk assessments. Up to recent years, PAHs were included in the National Control Plan for Animal Feed, but there is no legal obligation to monitor PAHs. The current (initial) risk assessment was undertaken to provide material to start the discussion on continued inclusion of PAHs in this National Control Plan.

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 National Control Plan for 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 National Control Plan 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 National Control Plan, whereas grass and mais 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 National Control Plan. If future data from

monitoring programs like these should be more suitable for risk assessment, the design of these programs should be changed.
To estimate the human exposure the milk consumption volume from EMEA (standard food basket for the safety assessment of veterinary medicinal products) was 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 National Control Plan 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 considered relevant. However, the higher calculated intake could still be of importance. Cancer of the gastro-intestinal tract is indeed seen in cows (personal comment RIKILT pathologist Dr. Maria Groot), 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 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 5 ng BaP/kg bw/day (0.5 ng/kg bw/day for total PAHs) 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 concentrations 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 National Control Plan for Animal Feed. 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.

4.14 Conclusions

4.14.1 Toxicology

- Data on non-carcinogenic effects are very limited. Similar applies on the oral carcinogenic potential of PAHs other than BaP.

4.14.2 Scenarios

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

4.14.3 Transfer from feed to animal products

- Data on transfer rates of PAHs to cows milk and other animal products are very limited;Metabolism is a factor 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 largely to the total concentration of PAHs in milk.

4.14.4 Human 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 Benzo(a)Pyrene up to a factor 2.75 for human consuming this milk. Based on the data in literature, this could be the result from carry-over of metabolites.

4.14.5 Animal exposure

- Calculated intake of PAHs by cows is 65 to 1000 times higher than the calculated intake by humans, while their weight is 4-8 times higher, which could indicate a potential risk for animal health.

4.14.6 National Control Plan for Animal Feed

- The current analysis of PAHs in food should not be expressed solely in Benzo(a)Pyrene equivalents;
- Analysis of PAHs in animal products should also include metabolites of PAHs;
- The design of the National Control Plan is not compatible with the use in risk assessment. Other feed ingredient categories should be included, such as the staple compound feed ingredient cereal, and fresh grass;
- In addition, the monitoring design should be adapted to provide a stronger statistical basis for risk assessment, by taking more samples for the animal feed categories other than oils/fats and dried roughage.

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5 Volatile Organic Compounds

5.1 Introduction

Volatile organic compounds (VOCs) are simple hydrocarbons which are liquid at room temperature and highly volatile, and classified by their boiling points (between 50˚ and 260˚ C, WHO definition). They originate from crude oil and derived products. In food production, they are used as solvents for extracting oil from oilseeds such as soy and rape, and flavourings from natural flavouring materials. For a specification of VOCs assessed in this chapter see table 1.

*Table 1: Name, log P value, CAS number and structural formula of VOCs assessed in this chapter. * BTEX is the abbreviation often used for the group of aromates Benzene, Toluene, Ethylbenzene and Xylene. ** The obtained log P values suggest an appreciable oral bioavailability (logP around 3) of most of these compounds, with the exception of acetone and ethylacetate.*

Compound	Log p**	Cas number	Structural formula
AROMATES (BTEX*)			
Benzene (BNZ)	2.13	71-43-2	 C6H6
Toluene (TOL) (methylbenzene)	2.73	108-88-3	 C7H8
Ethylbenzene (EBZ)	3.15	100-41-4	 C8H10
Ortho-xylene (OXL) (1,2 dimethylbenzene)	3.12	95-47-6	 C8H10
Meta-xylene (MXL) (1,3 dimethylbenzene)	3.2	108-38-3	 C8H10
Para-xylene (PXL) (1,4 dimethylbenzene)	3.15	106-42-3	 C8H10
ALKANES			
n-Pentane (PEN)	3.39	109-66-0	C5H12
n-Hexane (HEX)	3.9	110-54-3	C6H14

n-Heptane (HEP)	4.66	142-82-5	C7H16
n-Octane (OCT)	5.18	111-65-9	C8H18
SPECIFIC ALKANES			
1-Hexene (HXE)	3.39	592-41-6	C6H12
Cyclohexane (CHX)	3.44	110-82-7	 C6H12
OTHER			
Acetone (ACT)	-0.24	67-64-1	 C3H6O
Ethylacetate (EAC)	0.73	141-78-6	 C4H8O2

In Europe, the use of VOCs in food is regulated by Directives 88/344/EEC, 92/115/EEC, 94/52/EEC and 97/60/EEC. ANNEX VIII shows the list of VOCs which are currently allowed for use as extraction solvents in the production of foodstuffs and food ingredients.
In feed, the use of extraction solvents is regulated under Regulation 1831/2003 (additives for use in animal nutrition). In this regulation, the extraction solvents classify as processing aids under definition 2h: ‘’processing aids’ means any substance not consumed as a feeding stuff by itself, intentionally used in the processing of feeding stuffs or feed materials to fulfill a technological purpose during treatment or processing which may result in the unintentional but technologically unavoidable presence of residues of the substance or its derivatives in the final product, provided that these residues do not have an adverse effect on animal health, human health or the environment and do not have any technological effects on the finished feed’. In short: if the residues do not pose a risk for (end)consumers of the ingredients, any compound can be used during the production of animal feed. In this document, the potential risks related to the presence of residues of VOCs in feed ingredients, as measured in the National Control Plan for Animal Feed in previous years, were evaluated.

5.2 Occurrence of residues of VOCs in foods

The industry has an economical benefit from recovering solvents from the extracted foodstuffs as much as possible, and will thus refine of the oils and fats further after extraction from the seeds. In foods for human consumption, the occurrence of residues will usually be low as required by Directives 88/344/EEC, 92/115/EEC, 94/52/EEC and 97/60/EEC. Due to their volatile nature, remaining concentrations of extraction solvents in food commodities can be even further reduced due to contact with the air and processing steps that involve heating, however evaporation of VOCs from oil may be

hampered because of its high surface tension. Residues of extraction solvents in food are generally considered to be easily metabolised in vivo and thus not to be a problem for the safety of the consumer. More emphasis is put on the exposure to VOCs by inhalation of workers in the vegetable oil extraction industry.
In the case of animal feed and feed ingredients however, materials usually are not highly refined, and concentrations of extraction solvents in feed ingredients may be expected to be high.

Limited information on the occurrence of VOCs in food and commodities could be found. In a Swedish survey, acetone was found in concentration of 18-226 mg/l in cow's milk and 2 mg/kg in beans (unknown reference cited by Kloet, 2002). In human blood plasma background concentrations of 1-2 mg/l can be found, much higher concentrations can be observed during fasting; 45 mg/l in plasma has been recorded (unknown reference cited by Kloet, 2002). In humans, acetone is also endogenously produced during metabolisation of foods and in particular fats. In ruminants, acetone is produced in significant amounts during microbial fermentation in the fore stomach system (personal comment professor Fink-Gremmels, University of Utrecht).

Fleming-Jones and Smith (2003) reported the results of an study of US Food and Drug Administration (FDA) on VOCs in 70 foods, which included animal products, over a 5 year period in the USA (1996-2000). VOCs were found in at least one sample of all foods tested, although no single compound was found in each of the foods. The total amount of VOCs found in a single food item over the 5 year period ranged from 24 to 5328 µg/kg. In creamed corn (canned) the lowest concentrations were observed and in cheddar cheese the highest. Benzene was found in all but 2 food products, with concentrations ranging from 1 to 190 µg/kg. The highest concentration was found in fully cooked ground beef, the average concentration was 40 ppb in the 12 analysed samples of fully cooked ground beef. Benzene concentrations above 100 µg/kg were also seen in at least one sample each of cola (138 µg/kg). Benzene formation in soft drinks (and other products) can occur when ascorbic acid (vitamin C) and the preservative sodium benzoate react together. Interestingly, in primary products such as raw bananas (132 µg/kg), and cole slaw (102 µg/kg) also high concentrations of benzene were found. According to the study of Hattemer-Frey et al. (1990, cited by ATSDR, 2005) benzene in products of plant origin primarily results from air-to-leaf transfer.

Contradictory results have been found in eggs. In the ATSDR draft Toxicological profile on benzene, it was mentioned that in an US-EPA inventory from 1982 concentrations up to 2100 µg/kg were found in uncooked eggs and 500–1900 µg/kg in hard-boiled eggs. Other cited studies reported concentrations under 2 µg/kg (McNeal et al., 1993, cited by ATSDR, 2005).

The total concentration of benzene on exposed food crops consumed by humans was estimated to be 587 ng/kg (Hattemer-Frey et al. 1990, cited by ATSDR, 2005). In the EU Risk Assessment Report (EU RAR, Chemicals Bureau, 2003, draft) it was stated that 'Indirect exposure via the environment has been calculated for the uptake of benzene via ambient air, drinking water, vegetables, milk, and meat. For all scenarios the most relevant contribution to the total daily dose is the uptake via air (96 - > 99%). Drinking water and fish consumption contribute 0.1 - 2% to the exposure. All other sources of exposure (milk, meat and vegetables) can be regarded as not significant.'

5.3 Toxicological profile

Due to their volatile nature, most toxicological studies on VOCs focus on exposure via inhalation. In the toxicological profile of this initial risk assessment, the focus is on oral exposure. Kinetics were only described for benzene and hexane, being the most toxic compound and most abundant compound present in animal feed ingredients (see paragraph 5.5), respectively.

5.3.1 Aromates (BTEX)

An elaborate desription of alkanes is made in the risk assessment of mineral oil in animal feed ingredients (see chapter 3). In short, fractions of alkanes are identified by dividing aromatic and aliphatic hydrocarbons by structure and further subdividing on the basis of equivalent carbon number 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. The aromatic fraction EC5-9 was considered by the Total Petroleum Hydrocarbons Criteria Working Group, Toxicology Technical Action Group (TPHCWG) to contain ethylbenzene, toluene, styrene and o,m,p-xylene. Benzene was excluded due to its carcinogenic properties and is discussed separately. An RfD of 0.2 mg/kg bw/day was set by TPHCWG for the whole fraction (cited by RIVM, 2001)

5.3.1.1 Benzene

Consumption of foods or liquids containing high concentrations of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, tachycardia, coma, and death. The health effects that may result from the consumption of lower concentrations of benzene are not known (ATSDR, 2005). Benzene is classified as a carcinogen (IARC, 1987). Exposure to benzene has been associated with development of a particular type of leukaemia, denoted acute myeloid leukaemia (AML).

At present it is not know whether or not benzene can induce adverse human health effects after long-term oral exposure. In rats and mice, exposure to food or water contaminated with benzene was found to affect the haematopoietic and the immune system, furthermore it caused several types of cancer at 25 mg/kg bw/day, such as Zymbal gland carcinomas, oral cavity squamous cell papillomas and carcinomas, malignant lymphomas and Harderian gland adenomas. (ATSDR, 2005)

Kinetics

The toxicokinetics of benzene have been studied in both animals and humans. The key findings suggest that benzene is absorbed via all exposure routes (inhalation, dermal and oral) with inhalation as the most important route of exposure. After absorption, benzene is rapidly distributed, with the highest concentrations in fat and in lipid rich tissues compared to blood. In vivo, benzene is rapidly metabolized and the metabolites are excreted mainly in the urine following phase-II-conjugation. Formation of the ultimate toxic species of benzene requires oxidative metabolism of benzene, the metabolic pathway is comparable for humans and animals. The liver is the major site of benzene metabolism, but metabolism in the bone marrow may be associated with the haematotoxic and leukaemogenic effects of benzene (EU RAR, 2003, draft).
It has been suggested that benzene toxicity works via a multiple metabolite type of mechanism, meaning that not just one metabolite is responsible for benzene toxicity, but multiple metabolites are involved. (EU RAR, 2003, draft).

IARC classified benzene in 1987 as a human carcinogen class I. They found in three independent cohort studies evidence of an increased incidence of acute nonlymphocytic leukaemia in workers exposed to benzene via inhalation. Other studies in rats and mice showed carcinogenic effects after oral, inhalation and intraperitoneal exposure.

Reference limits
RIVM (2001): MPR 0.0033 mg/kg bw/day.
RIVM has derived in 2001 a provisional oral maximum permissible risk dose (MPR) of 0.0033 mg/kg bw/day. This value was derived from a human occupational cohort study, in which 748 were exposed to benzene via inhalation for at least one day over 9 years cohort study, using route-to-route extrapolation. The toxicological endpoint in the study was leukemia (RIVM, 2001).

WHO (1993, 2003): drinking water guideline value 0.01 mg/l.
WHO derived a guideline value of 0.01 mg/l in drinking water, using a linear extrapolation model (because of statistical lack of fit of derivation some of the data with the linearized multistage model) applied to leukaemia and lymphomas found in female mice, and oral cavity squamous cell carcinomas found in male rats in a 2-year gavage study in rats and mice (WHO, 2003, original assessment in 1993). The 1993 Guidelines estimated the range of benzene concentrations in drinking-water corresponding to an upper-bound excess lifetime cancer risk of 10^{-5} to be 0.01– 0.08 mg/l based on carcinogenicity in female mice and male rats. As the lower end of this estimate corresponds to the estimate derived from epidemiological data, which formed the basis for the previous guideline value of 0.01 mg/l associated with a 10-5 upper-bound excess lifetime cancer risk, the guideline value of 0.01 mg/l was retained. Due to the carcinogenicity of benzene, no TDI was set.

EPA (2005): RfD 0.004 mg/kg bw/day
US-EPA derived an oral reference dose (RfD) for benzene of 0.004 mg/kg bw/day, based on the results of BMD modelling of ALC data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m3 and adjusted from intermittent to continuous exposure (BMCLADJ=8.2 mg/m3). Route-to-route extrapolation methodology was applied to convert from inhalation to equivalent oral exposure, resulting in an equivalent oral dose rate of 1.2 mg/kg/day. This value was divided by a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies). EPA also classified benzene as carcinogenic, and derived from epidemiological studies the drinking water concentration of 1-10 µg/L at specified risk levels of 1 in 10^{-6} for leukaemia (2000):

IARC (1987): Carcinogenic group I.
IARC classified benzene in 1987 in group I; carcinogenic for humans.

The lowest reference value for oral exposure of 0.0033 mg/kg bw/day as derived by RIVM in 2001 was used in this risk assessment.

5.3.1.2 Toluene

Studies of the effects of oral exposure to toluene are limited. Only one study could be found regarding health effects in humans after oral exposure to toluene, and there are only a minimal number of animal studies. Adverse effects include cardiovascular, haematological, hepatic, and renal effects in animals exposed orally to toluene at dosage levels up to 2500 mg/kg/day for 13 weeks, or 590 mg/kg/day for 6

months (Hsieh et al., 1989; NTP, 1990; Wolf et al., 1956, cited by ATSDR, 2000). Oral exposure of rats or mice to toluene doses of 2500 mg/kg/day for 13 weeks was not found to induce any musculoskeletal, gastrointestinal or respiratory effects (NTP, 1990, cited by ATSDR, 2000).

Neither statistically significant respiratory, gastrointestinal, haematological, musculoskeletal effects, nor effects on the adrenal or thyroid glands, nor on the histology or weight of the spleen or thymus, were reported in mice or rats after oral exposure to toluene at dosage levels up to 2500 mg/kg/day for 13 weeks (NTP, 1990) or 650 mg/kg/day for 6 months (Wolf et al., 1956, cited by ATSDR, 2000).

Increased relative heart weight, increase in liver weight, increases in the relative kidney weights and neurological effects have been recorded after oral exposure (ATSDR, 2000).

Reference limits
WHO (1993, 2003): TDI 0.223 mg/kg bw/day.
WHO has derived a TDI of 0.223 mg/kg bw/day, based on a LOAEL of 312 mg/kg of body weight per day for marginal hepatotoxic effects observed in a 13-week gavage study in mice, correcting for 5 days per week dosing and using an uncertainty factor of 1000 (100 for inter- and intraspecies variation and 10 for the short duration of the study and use of a LOAEL instead of a NOAEL) (WHO, 2003, original assessment in 1993).

RIVM (2001): TDI 0.223 mg/kg bw/day.
RIVM has adopted the WHO (1993,1996) TDI of 0.223 mg/kg bw/day in 2001.

ATSDR (2000): MRL 0.02 mg/kg bw/day for intermediate duration exposure (15–364 days).
ATSDR did not derive an Minimum Risk Level (MRL) for chronic oral exposure as they found no suitable data available for toluene. An MRL of 0.02 mg/kg bw/day has been derived for an intermediate duration (15–364 days) of oral exposure to toluene. This MRL was derived from a LOAEL of 5 mg/kg/day based on regional increases in monoamine neurotransmitters in the brains of CD1 mice exposed to toluene through their drinking water for 28 days (Hsieh et al. 1990b, cited by ATSDR, 2000).

EPA (2005): RfD 0.08 mg/kg bw/day.
US-EPA has derived an RfD of 0.08 mg/kg bw/day in 2005, based on a BMDL of 238 mg/kg/day for increased kidney weight in rats.

The lowest RfD of 0.08 mg/kg bw/day as derived by EPA in 2005 was used in this risk assessment.

5.3.1.3 Ethylbenzene

There are no reliable data on the effects in humans after oral exposure to ethylbenzene. One oral intermediate-duration study in rats was found in the literature (Wolf et al. 1956), in which female rats were orally exposed to 13.6-680 mg/kg/body weight ethylbenzene by gavage for 6 months. Effects that were found were increased kidney and liver weight and histopathological changes in both organs. Some changes were also found in heamatological parameters. However, no conclusions could be drawn
from these results because of serious weaknesses in the methodology and reporting of the data (ATSDR, 1999).

The only available reproduction study with animals indicates that acute oral exposure to 500 or 1000 mg/kg ethylbenzene decreases peripheral hormone levels and may block or delay the estrus cycle in female rats during the diestrus stage (Ungvary, 1986, cited by ATSDR). Decreased levels of hormones, including luteinizing hormone, progesterone, and 17 β-estradiol, were accompanied by uterine changes. These consisted of increased stromal tissue with dense collagen bundles and reduced lumen. No dose response was noted. The study limitations included lack of rationale for dose selection, use of only two doses, small number of test animals, and no statistical analysis of the data (ATSDR, 1999).

In inhalation studies, ethylbenzene has been shown to have carcinogenic properties. An NTP-sponsored 2-year inhalation bioassay in rats revealed a significant increase in interstitial cell adenomas and bilateral testicular adenomas, but not mice at 750 mg/kg ethylbenzene (NTP, 1999). IARC (2000) concluded that ethylbenzene is possibly carcinogenic to humans (group 2B) based on inadequate evidence in humans and sufficient evidence in animals.

Reference limits
RIVM (2001): TDI 0.1 mg/kg bw/day.
RIVM derived a TDI of 0.1 mg/kg bw/day in 2001, based on the TDI assessment of 1991 (which resulted in a TDI of 136 µg/kg bw/day based on a NOAEL of 136 mg/kg bw/day for liver and kidney toxicity in rats). The 1991 TDI was adjusted for exposure duration (back calculation from the NOAEL for 5 days/week to the value for 7 days/week). The adjusted NOAEL was 97 mg/kg bw/day. Applying an UF of 1000 (as was done in 1991) resulted in a TDI of 100 µg/kg bw/day.

WHO (1993, 2003): TDI 0.097 mg/kg bw/day.
WHO has derived a TDI of 0.097 mg/kg of body weight/day, using the same studie and extrapolation factor as was done by RIVM (WHO, 2003, original assessment in 1993).

EPA (1991): RfD 0.1 mg/kg bw/day.
US-EPA has set an RfD of 0.1 mg/kg bw/day based on a NOEL of 97.1 mg/kg/day for increased weight and histopathological changes in the liver and kidneys of rats (EPA, 1991). Confidence in the RfD is low because in the study used for setting the RfD (Wolf et al., 1952)2, rats of only one sex were tested and the experiment was not of chronic duration. Confidence in the supporting database is low because other oral toxicity data were not found.

ATSDR (1999): no derived MRLs.
ATSDR derived no acute-, intermediate-, or chronic-duration oral MRLs for ethylbenzene due to lack of appropriate data (ATSDR, 1999).

IARC (2000): carcinogenic group 2B.
IARC classified in 2001 ethylbenzene as category 2B; possibly carcinogenic to humans based on sufficient evidence for the carcinogenicity in experimental animals (inhalation studies).

The adjusted TDI of 0.1 mg/kg bw/day from RIVM (2001) was used in this risk assessment.

2 The TDI of RIVM and WHO were based on the same study.

5.3.1.4 Xylenes

NTP found a 5–8% decrease in body weight gain (which they considered biologically not significant) and unexplained reduced survival rate in male F344 rats at 500 mg/kg (NTP, 1986). In a 90-day gavage study using xylenes and individual isomers mild nephropathy was found in female rats (Condie et al., 1988, cited by RIVM, 2001). Transient hyperactivity was noted after gavage administration in male and female B6C3F1 mice at a dose of 1000 mg/kg/day (NTP, 1986, cited by ATSDR, 2005).

Reference limits

RIVM (2001): TDI 0.15 mg/kg bw/day.

RIVM derived in 2001 a TDI of 0.15 mg/kg bw/day based on a LOAEL of 150 mg/kg bw/day for increased incidence of mild chronic nephropathy in female rats in a 90-day gavage study using mixed xylenes and individual isomers (Condie et al., 1988, cited by RIVM, 2001). The response at this dose was marginal only and it was considered that the NOAEL would be only slightly lower. The LOAEL was divided by an uncertainty factor of 1000, including inter- and intraspecies factors of 10, and an extra factor of 10 for limited duration of the pivotal study. Because of the mild nature of the effect seen at the LOAEL, the use of an extra factor for the use of a LOAEL was not considered necessary.

WHO (1993, 2003): TDI 0.179 mg/kg bw/day.

WHO derived a TDI of 0.179 mg/kg bw/day, based on a NOAEL of 250 mg/kg of body weight per day for decreased body weight in a 103- week gavage study in rats, correcting for 5 days per week dosing and using an uncertainty factor of 1000 (100 for inter- and intraspecies variation and 10 for the limited toxicological end-points) (WHO, 2003, original assessment in 1993).

ATSDR (2005): MRL 0.6 mg/kg/day.

ATSDR derived an MRL of 0.6 mg/kg/day for chronic oral exposure (≥1 year) to mixed xylenes.
A NOAEL of 500 mg/kg was identified for hyperactivity in mice, but this was not selected as the basis for the MRL, because that dose decreased the survival rate in male rats. Therefore, the rat NOAEL of 250 mg/kg was selected as the basis of the MRL. The NOAEL was first adjusted for discontinuous exposure (5 days/7 days), resulting in a duration-adjusted NOAEL of 179 mg/kg/day. An uncertainty factor of 100 (10 for extrapolation between animals and humans and 10 for human variability) and a modifying factor of 3 were applied to the duration-adjusted NOAEL to account for the lack of testing for sensitive neurological end points (the most sensitive effects in inhalation studies and acute oral studies). The resulting MRL of 0.6 mg/kg/day is considered to be protective to human health under the conditions of chronic oral exposure (>1 year) to mixed xylenes or individual isomers.

The lowest reference value of 0.15 mg/kg bw/day as derived by RIVM in 2001 was used in this risk assessment.

5.3.2 Alkanes

5.3.2.1 n-Hexane

It must be noted that the term ‘hexane’ is used for two types of solvents. First of all, there is the pure form of aliphatic alkane as illustrated in table 1, this type is usually referred to as n-hexane. Then there is the technical grade of hexane, a mixture of isomers of hexane. This latter type is mostly referred to as technical grade hexane, but the simple term ‘hexane’ is also used. In this document, we focus on the effects and reference value for n-hexane.

n-Hexane is known to exert peripheral neuropathy after oral exposure. Studies in rats showed that a metabolite of n-hexane (2,5-hexanedione) causes the neuronal damage, and not n-hexane itself (ATSDR, 1999).

A study in rats was conducted by Krasavage et al. in 1980 (cited by ATSDR, 1999) comparing oral (gavage) administration of n-hexane with its metabolites and technical grade hexane for 90-120 days. At 4,000 mg (46.2 mmol)/kg/day, clinical signs of neurotoxicity (severe hindlimb weakness or paralysis) were present after 101 days in 3 out of 4 rats. At this dose, also histological evidence of tibial nerve alterations (multifocal axonal swellings, adaxonal myelin infolding, paranodal myelin retraction) was observed. Clinical signs of neurotoxicity were seen with all of the metabolites at different timepoints at equimolar doses of 6.6 mmol/kg bw/day but not with practical grade hexane. Time to onset was as little as 16.8 days with 2,5-hexanedione. With practical grade hexane only one rat of 5 treated with 4,000 mg/kg/day showed histological lesions.

Kinetics
No studies were located that specifically addressed absorption of n-hexane after oral exposure in humans or animals. Absorption of n-hexane by the oral route in humans can be inferred from the appearance of n-hexane in exhaled air and 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al.1998, cited by ATSDR, 1999). Absorption of toxicologically significant amounts by this route can be inferred since neurological effects occurred in rats receiving n-hexane by gavage (Krasavage et al. 1980; Ono et al.1981, cited by ATSDR, 1999). Significant serum levels of the n-hexane metabolite 2,5-hexanedione were also measured in rats receiving n-hexane by gavage (Krasavage et al.1980, cited by ATSDR, 1999). No studies were located regarding distribution of n-hexane after oral exposure in humans or animals.
Little information is available on the metabolism of n-hexane after oral exposure, although it appears to be qualitatively similar to that after inhalation exposure. Metabolism of n-hexane takes place in the liver. When male Wistar rats were exposed via inhalation to n-hexane at concentrations up to 3,074 ppm for 8 hours, analysis of urine showed that 2-hexanol was the major metabolite, accounting for about 60-70% of the total metabolites collected over the 48-hour collecting period (Fedtke and Bolt 1987, cited by ATSDR, 1999). This is in contrast to humans, in which the major urinary metabolite is 2,5-hexanedione (Perbellini et al.1981, cited by ATSDR, 1999). Peak serum concentrations of the n-hexane metabolite 2,5-hexanedione of 24, 44, and 53 µg/rnL were observed in rats after a single gavage exposure to 570, 1,140, and 4,000 mg/kg n-hexane, respectively (Krasavage et al.1980, cited by ATSDR, 1999). Serum 2,5-hexanedione concentrations rose slowly to a peak at 12-16 hours and returned to baseline by 24 hours. Excretion of n-hexane after oral exposure in humans can be inferred from the appearance of n-hexane in exhaled air and 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al.1998, cited by ATSDR, 1999). No studies were located regarding excretion of n-hexane or n-hexane metabolites following oral exposure to n-hexane in animals (ATSDR, 1999).

Reference limits
EPA (2005): provisional RfD of 0.06 mg/kg bw/day.
US-EPA derived a provisional RfD of 0.06 mg/kg bw/d based on neurological and reproductive effects in rats. The provisional RfD is a value that has had some form of Agency review but is not on EPA's Integrated Risk Information System (IRIS, 2005).

ATSDR (1999): no derived limits.
ATSDR did not derive an MRL for oral exposure to n-hexane because of the limited database for oral exposure to n-hexane and the lack of toxicokinetic data for this route of exposure.

The provisional RfD of 0.06 mg/kg bw/day from EPA was used in this risk assessment.

5.3.3 Specific alkanes

5.3.3.1 1- Hexene

No information could be found on the oral toxicity of 1-hexene. Only few data are cited in the Hazardous Substances DataBank (HSDB), which indicate that when inhaled, 1-hexene at a concentration of about 0.1% produces CNS depression in humans, with accompanying vertigo, vomiting, and cyanosis. It is a low to moderate irritant to the skin, mucous membranes and eyes (Clayton & Clayton, 1993-94, cited by HSDB).

Reference limits
No reference limits were found.

5.3.3.2 Cyclohexane

No published data on oral repeated dose toxicity are available (EU RAR, 1999).

Some toxicological effects can be identified from inhalation studies. EPA cited two 90-day whole-body inhalation studies in mice and rats (DuPont, 1996, cited by EPA, 1993). In mice, clinical signs of hyperactivity and marked central nervous system stimulation were the main observed symptoms. The clinical observations of response to an auditory alerting stimulus varied as it was diminished in some instances and it could not be assessed due to hyperactivity at other periods. In addition, relative liver weights were increased. All symptoms diminished during a recovery period of one month. In rats, the most common clinical observation was diminished alerting responses in the chamber during exposure. This effect was characterized as transient and was not observed immediately after removing the animals from the chamber.

Reference limits
EPA: no derived limits.
US-EPA has evaluated the noncancer oral toxicity data for cyclohexane, but did not derive a reference dose (RfD) because no adequate oral exposure studies of humans or animals exist from which an oral RfD may be derived. EPA also determined that there are no adequate data for using route-to-route extrapolation from inhalation toxicity studies to derive an RfD.

5.3.4 Other VOCs

5.3.4.1 Acetone

Health effects from long-term oral exposures are known mostly from animal studies. Kidney, liver, and nerve damage, an increased incidence of birth defects, and lowered ability to reproduce (males only) occurred in animals following long-term exposure. However, these effects occurred at dosages above 500 mg/kg bw/day. It is not known if the same effects can occur in humans (ATSDR, 1994).
Direct toxicity of acetone to the digestive system could not be demonstrated. Histological examination of the gastrointestinal tract of rats and mice exposed to acetone in drinking water for 13 weeks (Dietz

et al. 1991; NTP 1991, cited by ATSDR, 1994) or of rats given acetone in water by gavage for 13 weeks (American Biogenics Corp., 1986, cited by ATSDR, 1994) did not reveal any treatment-related lesions. No studies have been conducted to assess the gastrointestinal effects in humans after oral (dietary) exposure to acetone. Only one incident of a man who intentionally drank approximately 200 mL of pure acetone (≈2241 mg/kg) and who had a red and swollen throat and erosions in the soft palate and entrance to the oesophagus has been described (Gitelson et al., 1966, cited by ATSDR, 1994).

It is worthwhile to mention that acetone may potentiate n-hexane neurotoxicity by decreasing body clearance of 2,5-hexanedione (Ladefoged and Perbellini, 1986, cited by ATSDR Toxicological profile on hexane, 1999)

Reference limits:
EPA (2003): RfD 0.9 mg/kg bw/day.
US-EPA derived in 2003 a reference dose of 0.9 mg/kg-day, based on nephropathy in rats following subchronic exposure via drinking water (Dietz et al., 1991; NTP, 1991), and using an uncertainty factor of 1000 (10 for intra-species variation, 3 for inter-species variation, 3 for extrapolation from subchronic studies, and 10 to account for database deficiencies).

5.3.4.2 Ethylacetate

Only one oral toxicity study could be found. US EPA sponsored in 1986 a 90-day subchronic study of ethyl acetate in rats. Four groups of rats (30/sex/group) were gavaged daily with 0, 300, 900 and 3600 mg/kg/day of ethyl acetate. Six weeks after the initial dosing, 10 rats/sex were subjected to interim sacrifice while the remaining rats continued on the dosing regimen until the final sacrifice (90 days). Male rats exposed to the high dose (3600 mg/kg/day) of ethyl acetate showed significant toxic effects, such as depressed body and organ weights, and depressed food consumption. Female rats exposed to the high dose showed slight but nonsignificant depression of above parameters compared with controls. The next lower dose (900 mg/kg/day) did not produce any adverse effects in either male or female rats and was therefore considered a NOEL.
Citations on inhalatory and dermal effects in the Hazardous Substances DataBank indicate that 'Ethyl acetate has…. reputation of being one of the least toxic of the volatile organic solvents'.

Reference limits
EPA (1986): RfD 0.9 mg/kg bw/day.
EPA derived in 1986 an RfD of 0.9 mg/kg bw/day based on a NOEL of 900 mg/kg bw/day for mortality and body weight loss in an oral subchronic study in rat. An uncertainty factor of 1000 was applied: 10 for intra- and 10 for interspecies extrapolation, and 10 to extrapolate subchronic to chronic exposure (EPA, 1986).

All RfDs and NOAELs/BMDs from which they were derived are summarized in table 2.

*Table 2: NOAELs from animal experiments from which reference values for humans were derived. NOAELs in mg/kg bw/day. All NOAELs were determined in rats, except for benzene, which is a route-to-route extrapolated BMDL from occupational studies (inhalation) in humans. * value is a BMDL. ** value is a LOAEL. ACT=Acetone, BNZ=Benzene, EAC=Ethylacetate, EBZ=Ethylbenzene, HEX=Hexane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.*

	ACT	BNZ	EAC	EBZ	HEX	TOL	OXL	M+PXL
RfD	0.9	0.003	0.9	0.1	0.06	0.08	0.15	0.15
NOAEL	900	1.2	900	97	4000	238*	150**	150**

5.4 Human oral exposure

No data was found on current levels of oral exposure of humans to VOCs. For the most toxic compound, benzene, it was concluded in the EU draft RA (2003) that 'inhalation is the dominant pathway for benzene exposure in humans, whereas oral and dermal exposure can be neglected'.

According to Environmental Health Perspectives (EHP, 1996), background concentrations for VOCs in human blood range from 70 ppt (styrene) to 1.1 ppb (toluene). This is appointed to non-occupational inhalation of VOCs, and it is indicated that smoking is an important source of exposure to aromatic VOCs.

5.5 Measurements in feed materials

Data from the National Control Plan for Animal Feed have been processed and results are shown in table 3. Matrices were categorised and maximum concentrations per category were determined. More details on the (processing of) data can be found in ANNEX IX Processing of VOC data.

Table 3: Maximum concentrationof VOCs in feed ingredient categories as measured in the National Control Plan for Animal Feed in the years 2000-2004. Concentrations in µg/kg. Feed ingredient categories are: 1=Flakes, 2a=Oils/fats-vegetable, 2b=Oils/fats-animal, 3=Citrus, 4a=Roughage-dried, 4b=Roughage-silage, 5=Other. – indicates that no data was available for this VOC in the feed ingredient category. ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

Feed ingredient category	ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+PXL
1	1700	-	890	-	-	16	10650	-	-	30	15	-	-
2a	6416	78	12411	2800	239	5359	42688	920	460	12140	2141	2400	2781
2b	3500	-	2029	117	-	958	3565	-	-	6382	-	503	3322
3	-	-	-	-	-	-	-	-	-	-	-	-	-
4a	-	-	-	-	-	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-	-	-	-	-	-
5	7650	-	-	-	-	-	1064	-	-	2634	-	-	-

5.6 Commodity limits

There are no EU- or national limits for the discussed VOCs in feed and feed ingredients. The limits for VOCs in foods can be found in Annex VIII.

5.7 Transfer from animal feed to products for human consumption

No data on carry-over of VOCs from feed to animal products were available.

The disposition of these compounds in animal tissues (residue formation) following oral exposure of farm animals with feed is expected to be limited, as all compounds are rapidly glucuronidated in the liver (pers. comm. Prof Fink-Gremmels). Clearance of VOCs from the body is thought to be a three-step process, with VOCs stored in fatty tissue to be cleared in the last phase (EHP, 1996). The latter indicates that storage in fat tissue might occur, which can result in intake of stored VOCs following consumption of animal fat.

5.8 Initial risk assessment

No data were available on carry-over of VOCs from feed to animal products. For evaluation of any potential risk for humans of VOCs present in feed, worst case scenarios were calculated.
For this, the measured maximum concentrations in feed ingredients were used to calculate a total intake of VOCs by food producing animals. This intake was estimated using feeding regimes and composition of compound feeds as described by Van Raamsdonk et al. (2006). Carry-over from this intake via feed was assumed to be 100% to each separate edible tissue in cow, pig and chicken. The intake of each VOC by humans via each separate edible tissue was calculated using the standard consumption parameters for the evaluation of veterinary drugs of the European Medicines Agency (EMEA, 2005). See for more details on the method used ANNEX VIII. The results of the calculations on intake via animal products from cattle, pigs and poultry are given in ANNEX X-ANNEX XII.

5.8.1 Human health

The results of the calculations showed that the identified toxicological safety limits were not exceeded by the calculated intake of VOCs via animal products even when 100% carry-over was assumed of maximum measured concentrations (see table 4 and 5 and ANNEX X to ANNEX XII). It should be noted that not for all VOCs measured in feed ingredients, toxicological reference values were identified. This conclusion can therefore only be applied to the VOCs mentioned in table 2 and 5. In addition, not all feed ingredient categories were included in the National Control Plan for Animal Feed (see ANNEX IX Processing of VOC data), resulting in a potential underestimation of the calculated intake of VOCs. On the other hand it needs to be stressed that the carry-over rate from VOCs is expected to be much lower than 100%, partly due to their fast metabolisation.

This conclusion might be less valid for the risk of carry-over of metabolites (especially relevant for the compounds hexane and benzene). As shown in the toxicological profile on hexane, the metabolite 2,5-hexanedione induces neurotoxic effects in rats at oral doses which are 7 times lower (based on mols) than its parent compound n-hexane (5 times based on weight). When the provisional RfD of 0.06 mg/kg bw/day is adjusted to 0.01 mg/kg bw/day based on this data, the ratio between the calculated hexane intake and this RfD might be less than 1 for some tissues such as milk, pig and chicken kidney and eggs (currently 1-10, see table 5). Even though an assumed carry-over of 100% for VOCs from feed to animal products is not realistic, the ratio of less than 1 between the adjusted provisional RfD an calculated intake for the metabolite 2,5-hexanedione indicates that special attention might be given to the possible carry-over of metabolites of VOCs.

From the results of the worst case calculations it is shown that based on the available information on toxicology and carry-over, risk from VOCs present in animal feed cannot be fully excluded. For the carry-over of parent compounds for which toxicological reference values are available, no risk is expected, but attention should be given to the metabolites of some of the VOCs.

Table 4: Overview of calculated intakes of VOCs by humans per animal product. Results are based on an estimated carry-over of 100% from maximum measured concentrations of VOCs in animal feed to each separate animal product, and food intake standards from the EMEA Standard Food Basket (EMEA, 2005). Calculated intake and reference values are expressed in µg/kg bw/day. ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

		ACT	**BNZ**	**CHX**	**EAC**	**EBZ**	**HEP**	**HEX**	**HXE**	**OCT**	**PEN**	**TOL**	**OXL**	**M+ PXL**
	RfD/TDI	900	3.3		900	100		60				80	150	150
Cattle														
Milk	summer	2.1	< 0.1	1.0	< 0.1	< 0.1	< 0.1	11.8	< 0.1	< 0.1	0.2	< 0.1	< 0.1	< 0.1
	winter	7.2	< 0.1	3.4	< 0.1	< 0.1	0.1	40.0	< 0.1	< 0.1	0.6	0.1	< 0.1	0.1
Meat		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Fat		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Liver		0.3	< 0.1	0.1	< 0.1	< 0.1	< 0.1	1.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Kidney		1.2	< 0.1	0.6	< 0.1	< 0.1	< 0.1	6.6	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
Pig														
Meat		0.2	< 0.1	0.1	< 0.1	< 0.1	< 0.1	0.7	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
Fat		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Liver		0.8	< 0.1	0.4	< 0.1	< 0.1	0.1	3.5	< 0.1	< 0.1	0.4	< 0.1	< 0.1	0.2
Kidney		4.1	< 0.1	1.8	< 0.1	< 0.1	0.3	16.9	< 0.1	< 0.1	1.8	< 0.1	0.1	0.8
Chicken														
Meat		0.5	< 0.1	0.4	0.1	< 0.1	0.1	2.4	< 0.1	< 0.1	0.4	< 0.1	0.1	0.1
Fat	unknown													
Liver		2.5	< 0.1	1.9	0.3	< 0.1	0.6	11.2	0.1	< 0.1	1.9	0.2	0.3	0.6
Kidney		1.3	< 0.1	1.0	0.1	< 0.1	0.3	5.9	< 0.1	< 0.1	1.0	0.1	0.1	0.3
Egg		10.7	< 0.1	1.6	< 0.1	< 0.1	0.3	15.0	< 0.1	< 0.1	4.4	< 0.1	0.1	0.9

Table 5: Ratio between RfD and calculated intake by humans per compound per animal product. RfDs are given in µg/kg bw/day. Ratios are classified based on orders of magnitude of 10. Only VOCs for which a reference value is derived are presented. Intake via chicken fat could not be calculated because of unknown fat percentage. Ratios for benzene and ethylbenzene in egg could not be calculated since data were only available in vegetable oil, which is not used as ingredient for compound feed for chickens (Raamsdonk et al., 2006). ACT=Acetone, BNZ=Benzene, EAC=Ethylacetate, EBZ=Ethylbenzene, HEX=Hexane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

		ACT	**BNZ**	**EAC**	**EBZ**	**HEX**	**TOL**	**OXL**	**M+PXL**
	RfD/TDI	900	3.3	900	100	60	80	150	150
Cattle									
Milk	summer	100 - 1000	> 1000	> 1000	> 1000	1 - 10	> 1000	> 1000	> 1000
	winter	100 - 1000	> 1000	> 1000	> 1000	1 - 10	> 1000	> 1000	> 1000
Meat		> 1000	> 1000	> 1000	> 1000	100 - 1000	> 1000	> 1000	> 1000
Fat		> 1000	> 1000	> 1000	> 1000	100 - 1000	> 1000	> 1000	> 1000
Liver		> 1000	> 1000	> 1000	> 1000	10 - 100	> 1000	> 1000	> 1000
Kidney		100 - 1000	> 1000	> 1000	> 1000	1 - 10	> 1000	> 1000	> 1000
Pig									
Meat		> 1000	> 1000	> 1000	> 1000	10 - 100	> 1000	> 1000	> 1000
Fat		> 1000	> 1000	> 1000	> 1000	100 - 1000	> 1000	> 1000	> 1000
Liver		> 1000	> 1000	> 1000	> 1000	10 - 100	> 1000	> 1000	100 - 1000
Kidney		100 - 1000	> 1000	> 1000	> 1000	1 - 10	> 1000	> 1000	100 - 1000
Chicken									
Meat		> 1000	> 1000	> 1000	> 1000	10 - 100	> 1000	> 1000	> 1000
Fat	unknown								
Liver		100 - 1000	100 - 1000	100 - 1000	100 - 1000	1 - 10	100 – 1000	100 - 1000	100 - 1000
Kidney		100 - 1000	100 - 1000	100 - 1000	100 - 1000	10 - 100	100 – 1000	100 - 1000	100 - 1000
Egg		10 - 100	-	100 - 1000	-	1 - 10	100 – 1000	100 - 1000	100 - 1000

5.8.2 Animal health

To obtain an indication on the risk of VOCs in feed for farm animals, the intake per animal species (based on maximum measured concentrations VOCs in feed ingredients) was taken from the worst case scenarios (see human health). The calculated intakes were then compared to the NOAELs in experimental animals from which the reference values for humans were derived. These NOAELs are summarized in table 2.

The ratios between NOAELs and calculated intake were determined (table 6). The results show that the NOAELs are not exceeded, but in some cases ratios are less than 100. This value is usually applied as uncertainty factor for inter- and intraspecies variation in the derivation of a toxicological reference value. This indicates that if farm animals are more sensitive to the toxic effects of VOCs, a animal health problem might occur. However, it should be noted that the NOAELs are determined from

chronic toxicity studies, which might be less relevant in the case of farm animals. Contamination of animal feed can be expected to occur batch-wise. Feeding of a contaminated batch may thus result in a semi-chronic exposure.

Table 6: Ratio between NOAELs and calculated intake by farm animals per compound. Ratios are classified in orders of magnitude of 10. Only VOCs for which a reference value is derived are presented. Ratios for benzene and ethylbenzene for egg-laying chickens could not be calculated since data were only available in vegetable oil, which is not used as ingredient for compound feed for chickens (Raamsdonk et al., 2006). ACT=Acetone, BNZ=Benzene, EAC=Ethylacetate, EBZ=Ethylbenzene, HEX=Hexane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

		ACT	BNZ	EAC	EBZ	HEX	TOL	OXL	M+PXL
Cattle	milk summer	100 - 1000	>1000	>1000	>1000	100 - 1000	>1000	>1000	>1000
	milk winter	10 - 100	>1000	>1000	>1000	10 - 100	>1000	>1000	100 - 1000
	meat	100 - 1000	>1000	>1000	>1000	100 - 1000	>1000	>1000	>1000
Pig		10 - 100	>1000	>1000	>1000	10 - 100	>1000	100 - 1000	10 - 100
Chicken	meat	10 - 100	1 - 10	100 - 1000	100 - 1000	10 - 100	10 - 100	10 - 100	10 - 100
	egg	10 - 100	-	>1000	-	10 - 100	>1000	100 - 1000	10 - 100

Formation of reactive metabolites from the parent compounds, may pose a risk for the food producing animals. The half life of the VOCs benzene, toluene and styrene have been shown to be less than 24 hours (Brugnone et al., 1986, 1992, 1993, cited by Ashley et al, 1996), indicating that production of VOC metabolites occurs rapidly after ingestion of VOCs.

5.9 Discussion

The Dutch Food and Consumer Product Safety Board (VWA) has the incentive to include certain contaminants in the yearly National Control Plan for Animal Feed (Regulation 882/2004), if risk assessments give rise to concern. Up to recent years, VOCs were included in the National Control Plan, but there is no legal obligation to monitor VOCs. The current (initial) risk assessment was undertaken to provide material for the discussion on whether or not to include VOCs in this National Control Plan.

For this risk assessment, VOCs can be categorised in aromates (BTEX), alkanes (regular and specific) and other compounds (acetone and ethylacetate). From the toxicological profile, it can first be concluded that not for all VOCs, an oral reference value is available. Due to their volatile nature, mostly inhalation studies are performed on VOCs, while in this risk assessment, oral data are relevant. The lack of oral date is additionally illustrated by the fact that some of the oral reference values are derived by route-to-route extrapolation from inhalation data. Due to the limited availability of oral toxicological studies, not on all VOCs measured in feed ingredients a full risk assessment could be performed.
It can be concluded that of the compounds for which oral toxicological studies were available, benzene and to a lesser extend ethylbenzene, is the most toxic compound. It is classified by IARC as carcinogenic to humans, based on induced leukaemia via toxicity to bone marrow.
The compound which is present in the highest concentration in feed ingredients is hexane (during the years 2001-2004). It is this compound that is the most commonly used extraction solvent for vegetable oil. The measured concentrations of hexane in the National Control Plan for Animal Feed reached up to 42 mg/kg in soy oil.

Although no data on carry-over from feed to animal products are available, some concentrations in animal tissues or products of animal origin in food have been found. In a US survey (Fleming-Jones et al. 2003), concentrations in the range from 24 to 5328 ppb (µg/kg) were found in 70 foods, including animal products. The source of the VOCs was not identified. In the EU draft Risk Assessment (2003) it was concluded that 'Drinking water and fish consumption contribute represent 0.1 - 2% to the total exposure. All other sources of exposure (milk, meat and vegetables) can be regarded not significant.'

5.9.1 Human health

The results from our initial risk assessment support this conclusion. It should be noted that not all feed ingredient categories were included in the monitoring program. Using the limited data, a worst case scenario assuming 100% carry-over of VOCs from feed to single animal products was used to calculate potential intakes. For all measured VOCs in feed, including the most toxic and most present (benzene and hexane), risks for human health effects were not expected. The calculated intake using the maximum concentrations measured in the National Control Plan for Animal Feed resulted in intakes far below the RfDs. Even the calculated concentrations of benzene in milk (0.0024 and 0.0082 µg/kg in summer and winter respectively, see ANNEX II) did not exceed the range of 1–10 µg/l in drinking water for 10^{-6} carcinogenic risk as derived by US-EPA in 2000.
Additional to this low calculated risk using 100% carry-over, it is not very likely that carry-over of parent compounds from feed to animal products occurs, due to rapid metabolism of the compounds. However, it is not known to what extent (toxic) metabolites are formed and carried over to animal products, excretion via milk might be a possibility. The ingestion of metabolites from VOCs might be especially relevant in the case of benzene, ethylbenzene and hexane, because the metabolites of these compounds are regarded as the major cause of the corresponding toxicity. In the case of hexane, adjusting the provisional RfD to the higher toxicity of its metabolite 2,5-hexanedione, results in a ratio of less than 1 between the calculated intake and this RfD for some animal products such as milk, pig and chicken kidney and eggs.

5.9.2 Animal health

To assess a possible risk from the measured VOCs in feed ingredients for food producing animals, the ratios between NOAELs for toxicity in rats and the calculated intake by farm animals were determined. In some cases, these ratios were less than 100. It should be noted that NOAELs derived from chronic toxicity studies may be less relevant for food producing animals. Considering however, that nothing is known about the sensitivity of farm animals as compared to laboratory experimental animals, these ratios indicate that animals health effects from the VOCs in feed ingredients cannot be excluded.
In addition, some VOCs, like pentane, are known to be toxic to paunch flora in cattle, resulting in disturbed digestion and reduced uptake of feed (pers. comm. Prof. Fink-Gremmels). A possible side effect of the concentrations of VOCs present in feed materials could also be an adverse taste, resulting in a reduced feed consumption (and weight gain losses).

The concentrations of VOCs are measured in crude animal feed ingredients. Due to the volatile nature of the compounds, it can be expected that concentrations present in feed materials are reduced during processing of the materials for the production of compound feed, reducing any possible risk of intake for animals or humans. In order to be able to make a more accurate intake assessment, it might be useful to measure VOCs in compound feed.

5.9.3 Regulating VOCs

This risk assessment does bring a complicated issue to light. In regulation 1831/2003 (additives for use in animal nutrition) the extraction solvents classify as processing aids. This means that compounds can be used in the production of animal feed, as long as residues in feed do no pose a risk for animals or humans. However, if the latter cannot be assessed because of lack of data on toxicology and/or carry-over from feed to animal products, a risk cannot be excluded. This lack of proof for negative effects on humans or farm animals can result in current practice that compounds are being used, and thus may pose an unidentified risk. As regards processing aids, the question may therefore be posed if the Regulation is fully effective in protecting human and/or animal health.

5.10 Conclusions and recommendations

- Of all measured VOCs in the National Control Plan for Animal Feed, benzene is the most toxic compound and hexane is the most abundant compound;

- A worst case scenario using 100% carry-over of maximum measured concentrations of VOCs from feed to animal products does not result in calculated intake by humans above known reference limits. Risk from intake of the resulting metabolites cannot be excluded;

- A quantitative risk assessment for food producing animals indicates that animal health effects may occur;

- Due to the fact that VOCs concentrations in feed ingredients might be reduced during processing of compound feed, it may be useful to measure concentrations in compound feed as end product;

- In absence of oral toxicity data, the (lack of) risk of the use of processing aids according to regulation 1831/2003 cannot be assessed;

- Based on the worst case scenario, inclusion of VOCs in the National Control Plan for Animal Feed is not recommended. The carry-over of possible metabolites to animal products remains a point of interest.

5.11 Acknowledgements

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ANNEX I Processing of PAH data

1. PAH concentrations have been measured in several feed ingredients in the years 2000-2004;
2. These ingredients were categorised;
3. Maximums and medians were calculated per category;
4. 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);
5. 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;
6. 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);
7. Using these transfer rates, maximum PAH concentrations in milk were calculated for several scenarios;
8. Human intake is calculated using the standard food basket (1.5 l milk/day) from EMEA;
9. 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).

Table A1: Specification of feed ingredients per category.

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

Ad 2- Maxima and medians of PAH concentrations measured in the feed ingredients in the National Control Plan for Animal Feed are specified in tables A2 and A3.

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	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	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	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	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	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 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.

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

In the National Control Plan for Animal Feed, 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.

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.

	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

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 National Control Plan for Animal Feed, 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.

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

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

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 mais 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.

*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.*

	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

Ad 5- Transfer rates of PAHs from feed to milk were taken from literature (see table A7). 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.

Table A7: 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.

		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	1.35						0.05		3.21	0.44		0.39
	Metabolites not done												
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

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 ANNEX II-ANNEX VII..

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.

ANNEX II PAH 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 concentrations 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 National Control Plan 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 ratio between the reference value and the calculated intake is given.
* 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	34	122	117	126	57	42	15	358	102	79	155	1370
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)	2.2	0.6	0.2	0.2	0.3	0.1	0.0	0.0	11.5	1.9	0.2	2.9	20.3
ug/l PAH in milk (27l)	0.1	0.0	0.0	8.7E-03	0.0	0.0	0.0	0.0	0.4	0.1	0.0	0.1	0.8
Human intake (1,5 l milk)	0.1	0.0	0.0	1.3E-02	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.2	1.1
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	2.2E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04*	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	29413			0.6					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	32	114	106	70	55	32	15	321	91	74	102	1054
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	11.510
PAHs transferred (in ug)	0.6	0.6	0.2	2.1E-01	0.1	0.1	0.0	0.0	10.3	1.7	0.1	1.9	16.0
ug/l PAH in milk (27l)	0.0	0.0	0.0	7.8E-03	0.0	0.0	0.0	0.0	0.4	0.1	0.0	0.1	0.6
Human intake (1,5 l milk)	0.0	0.0	0.0	1.2E-02	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.1	0.9
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	2.0E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	113252			0.7					4193	24957		16724	

ANNEX III PAH 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 concentrations 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 National Control Plan 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 ratio between the reference value and the calculated intake is given.
* 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)	236	100	159	128	132	84	127	58	815	1022	98	728	3687
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	####
PAHs transferred (in ug)	3.2	1.9	0.3	0.3	0.3	0.2	0.1	0.1	26.2	19.4	0.2	13.8	65.9
ug/l PAH in milk (27l)	0.1	0.1	0.0	9.5E-03	0.0	0.0	0.0	0.0	1.0	0.7	0.0	0.5	2.4
Human intake (1,5 l milk)	0.2	0.1	0.0	1.4E-02	0.0	0.0	0.0	0.0	1.5	1.1	0.0	0.8	3.7
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	2.4E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	20378			0.6					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)	42	29	75	68	52	38	45	15	330	102	52	113	961
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)	0.6	0.6	0.2	1.4E-01	0.1	0.1	0.0	0.0	10.6	1.9	0.1	2.1	16.4
ug/l PAH in milk (27l)	0.0	0.0	0.0	5.1E-03	0.0	0.0	0.0	0.0	0.4	0.1	0.0	0.1	0.6
Human intake (1,5 l milk)	0.0	0.0	0.0	7.6E-03	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.1	0.9
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	1.3E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	115028			1.1					4080	22202		15094	

ANNEX IV PAH 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 concentrations 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 National Control Plan 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 ratio between the reference value and the calculated intake is given.
* 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)	308	166	195	140	139	110	213	101	1273	1943	116	1301	6004
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	####
PAHs transferred (in ug)	4.2	3.2	0.4	2.8E-01	0.3	0.2	0.1	0.2	40.9	36.9	0.2	24.7	111.5
ug/l PAH in milk (27l)	0.2	0.1	0.0	1.0E-02	0.0	0.0	0.0	0.0	1.5	1.4	0.0	0.9	4.1
Human intake (1,5 l milk)	0.2	0.2	0.0	1.6E-02	0.0	0.0	0.0	0.0	2.3	2.1	0.0	1.4	6.2
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	2.6E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	15590			0.5					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	26	36	31	35	20	58	15	339	114	30	124	868
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)	0.6	0.5	0.1	6.2E-02	0.1	0.0	0.0	0.0	10.9	2.2	0.1	2.4	16.8
ug/l PAH in milk (27l)	0.0	0.0	0.0	2.3E-03	0.0	0.0	0.0	0.0	0.4	0.1	0.0	0.1	0.6
Human intake (1,5 l milk)	0.0	0.0	0.0	3.4E-03	0.0	0.0	0.0	0.0	0.6	0.1	0.0	0.1	0.9
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	5.7E-05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	116861			2.4					3972	19995		13754	

ANNEX V PAH 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 National Control Plan for Animal Feed. 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 National Control Plan 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 National Control Plan), 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 ratio between the reference value and the calculated intake is given.

* 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	
PAH intake cow (in ug)	16	15	58	55	35	29	12	8	107	35	38	36	0
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)	0.2	0.3	0.1	1.1E-01	0.1	0.1	0.0	0.0	3.4	0.7	0.1	0.7	0.0
ug/l PAH in milk (27l)	0.0	0.0	0.0	4.1E-03	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Human intake (1,5 l milk)	0.0	0.0	0.0	6.1E-03	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	1.0E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	309353			1.4					12631	64975		47605	

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	
PAH intake cow (in ug)	52	15	61	58	64	30	15	8	117	38	40	52	549
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)	0.7	0.3	0.1	1.2E-01	0.1	0.1	0.0	0.0	3.8	0.7	0.1	1.0	7.0
ug/l PAH in milk (27l)	0.0	0.0	0.0	4.3E-03	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.3
Human intake (1,5 l milk)	0.0	0.0	0.0	6.5E-03	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.4
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	1.1E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	92584			1.3					11455	59685		33087	

ANNEX VI PAH 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 National Control Plan for Animal Feed. 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 National Control Plan 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 National Control Plan for Animal Feed), 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 ratio between the reference value and the calculated intake is given.

* 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	
PAH intake cow (in ug)	56	19	65	62	68	34	19	12	390	182	44	301	1251
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)	0.8	0.4	0.1	1.2E-01	0.1	0.1	0.0	0.0	12.5	3.5	0.1	5.7	23.4
ug/l PAH in milk (27l)	0.0	0.0	0.0	4.6E-03	0.0	0.0	0.0	0.0	0.5	0.1	0.0	0.2	0.9
Human intake (1,5 l milk)	0.0	0.0	0.0	6.9E-03	0.0	0.0	0.0	0.0	0.7	0.2	0.0	0.3	1.3
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	1.1E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	86030			1.2					3448	12491		5659	

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.

ANNEX VII PAH 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 National Control Plan for Animal Feed. 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 addition, the ratio between the reference value and the calculated intake is given.

* 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
PAH intake cow (in ug)	165	148	221	173	187	128	163	96	891	1623	136	1026	4957
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)	2.2	2.8	0.4	0.3	0.4	0.3	0.1	0.2	28.6	30.8	0.3	19.5	85.9
ug/l PAH in milk (25l)	0.1	0.1	0.0	1.4E-02	0.0	0.0	0.0	0.0	1.1	1.2	0.0	0.8	3.4
Human intake (1,5 l milk)	0.1	0.2	0.0	2.1E-02	0.0	0.0	0.0	0.0	1.7	1.9	0.0	1.2	5.2
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	3.5E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	26999			0.4					1399	1297		1539	

Results based on median PAH concentrations in feed:

	ACE	ACY	BAA	BAP	BBF	BKF	CHR	DBA	PHE	FLU	IDP	PYR	Total PAH
PAH intake cow (in ug)	22	31	92	89	65	53	39	23	124	78	67	65	749
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)	0.3	0.6	0.2	1.8E-01	0.1	0.1	0.0	0.0	4.0	1.5	0.1	1.2	8.4
ug/l PAH in milk (25l)	0.0	0.0	0.0	7.1E-03	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.3
Human intake (1,5 l milk)	0.0	0.0	0.0	1.1E-02	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.1	0.5
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	1.8E-04	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RfD ug/kg bw/day	60	-	-	1.4E-04	-	-	-	-	40	40	-	30	
Ratio RfD/intake human	203378			0.8					10014	26909		24279	

ANNEX VIII VOCs allowed in food production

List of VOCs which are currently allowed for use as extraction solvents in the production of foodstuffs and food ingredients following Directives 88/344/EEC, 92/115/EEC, 94/52/EEC and 97/60/EEC. The tables are taken from the Proposal for a directive of the European Parliament and of the Council on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients (Codified version, COM[2003] 467 final 2003/0181 [COD]).

Part I Extraction solvents to be used in compliance with good manufacturing practice for all uses[1].

Propane Butane Ethyl acetate Ethanol Carbon dioxide Acetone → 1 2 ← Nitrous oxide	↓ 88/344/EEC (adapted) → 1 92/115/EEC Art. 1 pt. 3 → 2 97/60/EC art. 1 pt. 2 → 3 94/52/EC Art. 1

Part II Extraction solvents for which conditions of use are specified

Name	Conditions of use (summary description of extraction)	Maximum residue limits in the extracted foodstuff or food ingredient
→ 2 Hexane[3] ←	→ 2 Production or fractionation of fats and oils and production of cocoa butter ←	→ 2 1 mg/kg in the fat or oil or cocoa butter ←
	→ 2 Preparation of defatted protein products and defatted flours ←	→ 2 10 mg/kg in the food containing the defatted protein products and the defatted flours ←
		→ 2 30 mg/kg in the defatted soya products as sold to the final consumer ←
	→ 2 Preparation of defatted cereal germs ←	→ 2 5 mg/kg in the defatted cereal germs ←
Methyl acetate	Decaffeination of, or removal of irritants and bitterings from coffee and tea	20 mg/kg in the coffee or tea
	Production of sugar from	1 mg/kg in the sugar

[1] An extraction solvent is considered as being used in compliance with good manufacturing practice if its use results only in the presence of residues or derivatives in technically unavoidable quantities presenting no danger to human health.

[2] The use of Acetone in the refining of olive- pomace oil is forbidden.

	molasses	
Ethylmethylketone → 1 [4] ←	Fractionation of fats and oils	5 mg/kg in the fat or oil
	Decaffeination of, or removal of irritants and bitterings from coffee and tea	20 mg/kg in the coffee or tea
→ [1] Methanol ←	→ [1] For all uses ←	→ [1] 10 mg/kg ←
→ [1] Propan-2-ol ←	→ [1] For all uses ←	→ [1] 10 mg/kg ←

Part III Extraction solvents for which conditions of use are specified (preparation of flavourings from natural flavouring materials)

Name	Maximum residue limits in the foodstuff due to the use of extraction solvents in the preparation of flavourings from natural flavouring materials	
Diethyl ether	2 mg/kg	
Hexane → 1 [5] ←	1 mg/kg	
→ 3 Cyclohexane ←	→ 3 1 mg/kg ←	
Methyl acetate	1 mg/kg	
Butan-1-ol	1 mg/kg	
Butan-2-ol	1 mg/kg	
Ethylmethylketone → 1 [5] ←	1 mg/kg	
Dichloromethane	→ 1 0,02 mg/kg ←	
→ 1 Propan-1-ol ←	→ 1 1 mg/kg ←	
→ 2 1,1,1,2-tetrafluoroethane ←	→ 2 0,02 mg/kg ←	

4

3 Hexane means a commercial product consisting essentially of acyclic saturated hydrocarbons containing six carbon atoms and distlling between 64 °C and 70 °C.

→ 1 The combined use of Hexane and Ethylmetylketone is forbidden. ←

4 1 The presence of n-Hexane in this solvent should not exceed 50 mg/kg. This solvent may not be used in combination with Hexane. ←

5 → The combined use of these two solvents is forbidden. ←

ANNEX IX Processing of VOC data

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

1. These ingredients were categorised;
2. Maximum concentrations were calculated per category;
3. These maximum concentrations were then used to calculate possible VOC concentrations in mixed feed for each food producing animal using the composition of mixed feed as provided by Kemme and Van Raamsdonk (2004);
4. With the calculated maximum concentrations in mixed feed, a maximum VOC intake for cattle, pig and chickens was calculated in end-of-breed scenarios of feed consumption;
5. Transfer rates of VOCs from feed to milk, meat, fat, liver, kidney and egg were assumed to be 100%;
6. Using these transfer rates, maximum VOC concentrations in foods were calculated;
7. Human intake is calculated using the standard food basket (1.5 l milk/day, 0.3 kg meat/day, 0.05 kg fat/day, 0.1 kg liver/day, 0.05 kg pork kidney/day, 0.01 chicken kidney/day and 0.1 kg eggs/day) and standard body weight of 60 kg from EMEA;
8. Calculated human intake is compared to the known toxicological reference values.

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

Ad 1- Categories differ in number of samples from which the results were used (see table A1).

Table A1: Specification of feed ingredients per category.

Category	Matrix	# samples (n)
1-Flakes	Soy	19
	Sunflower	11
	Reap	10
	Sesame	1
	Palm seed	1
	Unknown vegetable origin	1
2a-Oils/fats-vegetable	Soy	13
	Palm seed	15
	Coconut	4
	Unknown vegetable origin	3
2b- Oils/fats- animal	Unknown animal origen	6
3-Citrus		None
4a-Roughage-dried		None
4b-Roughage-silage		None
5-Other	Soy lecithin	2

Ad 2- Details of VOC concentrations measured in the feed ingredients in the National Control Plan for Animal Feed are specified in tables A2, A3 and A4. Only results above the Limit of Detection (LOD) were used in the risk assessment.

Table A2: Statistics of results of all samples as measured in the National Control Plan for Animal Feed in the years 2000-2004. Positive result is a result above LOD. Concentrations in µg/kg. ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

		ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+ PXL
min	ug/kg	16	18	0	11	36	16	11	920	460	0	12	30	35
max	ug/kg	7650	78	12411	2800	239	5359	42688	920	460	12140	2141	2400	3322
med	ug.kg	449	38	160	41	60	245	1012	920	460	1400	45	176	978
No of samples		199	199	199	199	199	199	199	199	199	199	199	199	199
# positives		48	6	39	10	5	24	60	1	1	25	13	13	6
% positives		24	3	20	5	3	12	30	1	1	13	7	7	3

Table A3: Maximum concentrations in feed ingredient categories as measured in the National Control Plan for Animal Feed in the years 2000-2004. Concentrations in µg/kg. Feed ingredient categories are: 1=Flakes, 2a=Oils/fats-vegetable, 2b=Oils/fats-animal, 3=Citrus, 4a=Roughage-dried, 4b=Roughage-silage, 5=Other. – indicates that no data was available for this VOC in the feed ingredient category. ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

Feed ingredient category	ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+ PXL
1	1700	-	890	-	-	16	10650	-	-	30	15	-	-
2a	6416	78	12411	2800	239	5359	42688	920	460	12140	2141	2400	2781
2b	3500	-	2029	117	-	958	3565	-	-	6382	-	503	3322
3	-	-	-	-	-	-	-	-	-	-	-	-	-
4a	-	-	-	-	-	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-	-	-	-	-	-
5	7650	-	-	-	-	-	1064	-	-	2634	-	-	-

Table A4: Median concentrations in feed ingredient categories as measured in the National Control Plan for Animal Feed in the years 2000-2004. Concentrations in µg/kg. Feed ingredient categories are: 1=Flakes, 2a=Oils/fats-vegetable, 2b=Oils/fats-animal, 3=Citrus, 4a=Roughage-dried, 4b=Roughage-silage, 5=Other. – indicates that no data was available for this VOC in the feed ingredient category. ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

Feed ingredient category	**ACT**	**BNZ**	**CHX**	**EAC**	**EBZ**	**HEP**	**HEX**	**HXE**	**OCT**	**PEN**	**TOL**	**OXL**	**M+ PXL**
1	327	-	158	-	-	16	1450	-	-	14	15	-	-
2a	220	38	1200	38	60	209	801	920	460	1600	128	153	288
2b	1100	-	2029	117	-	410	390	-	-	1400	-	503	3322
3													
4a													
4b													
5	4036	-	-	-	-	-	821	-	-	2634	-	-	-

Ad 3- The contribution of individual feed ingredient categories to compound feed of the food producing animals are given in ANNEX X - ANNEX XII and taken from Kemme en Van Raamsdonk, 2004. Not all ingredients for compound feed were represented in the samples from the National Control Plan for Animal Feed, explaining why the sum of the percentages of contribution of individual feed ingredient categories is less than 100%. It may be possible that VOCs in the ingredients not represented here might have added to the calculated VOC content of compound feed. For this initial risk assessment it is assumed that all VOCs in the concentrate originate only from the feed ingredients listed in the respective Annexes.

ANNEX X Calculated intake VOCs - cattle

Calculated carry-over and intake of VOCs via **cattle** using maximum measured concentrations in feed ingredients.

Feeding regime	Summer		Winter	
	kg	% total	kg	% total
Fresh grass	7.9	47.9		
Grass silage	4.3	26.1	7.5	39.5
Corn silage	2.2	13.3	4.4	23.2
Compound feed	2.1	12.7	7.1	37.4
Total	16.5		19	

	Percentage of feeding regime	
category	summer	winter
1	7.21	21.17
2a	0.01	0.01
2b	0.04	0.12
5	0.21	0.61

Intake via **milk**

Summer	**ACT**	**BNZ**	**CYC-HEX**	**ETH-ACE**	**ETH-BNZ**	**HEP**	**HEX**	**1-HEXE**	**OCT**	**PEN**	**TOL**	**O-XYL**	**M+P-XYL**
PAH intake cow (in ug)	2312	0	1083	3	0	30	12764	1	0	180	20	6	25.36
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	101.00
PAHs transferred (in ug)	2312	0	1083	3	0	30	12764	1	0	180	20	6	25.61
ug/l PAH in milk (27l)	85.6	0.0	40.1	0.1	0.0	1.1	472.8	0.0	0.0	6.7	0.7	0.2	0.95
Human intake (1,5 l milk)	128.4	0.0	60.2	0.2	0.0	1.7	709.1	0.0	0.0	10.0	1.1	0.3	1.423
ug/kg bw (60 kg EMEA)	2.1	0.0	1.0	0.0	0.0	0.0	11.8	0.0	0.0	0.2	0.0	0.0	0.024
Winter													3.21
PAH intake cow (in ug)	7817	0	3662	11	1	102	43155	3	1	608	66	19	4.811
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	0.080
PAHs transferred (in ug)	7817	0	3662	11	1	102	43155	3	1	608	66	19	86
ug/l PAH in milk (27l)	289.5	0.0	135.6	0.4	0.0	3.8	1598.4	0.1	0.0	22.5	2.5	0.7	3.2
Human intake (1,5 l milk)	434.3	0.0	203.4	0.6	0.0	5.7	2397.5	0.1	0.1	33.8	3.7	1.0	4.8
ug/kg bw (60 kg EMEA)	7.2	0.0	3.4	0.0	0.0	0.1	40.0	0.0	0.0	0.6	0.1	0.0	0.1

ACT=Acetone, BNZ=Benzene, CYC-HEX=Cyclohexane, ETH-ACE=Ethylacetate, ETH-BNZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, 1-HEXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, O-XYL=O-Xylene, M+P-XYL=M+P Xylene.

Intake via **meat and organs** of cattle, 16 months old, 500 kg

Feeding regime	kg	% total
Corn silage	4.7	68.1
Compound feed	2.2	31.9
Total	6.9	

Percentage of feeding regime	
Category	Percentage
1	18.06
2a	0.01
2b	0.11
5	0.52

Weigth of organs of cattle

Age	Weight	Organs (kg, %)			
months	kg	liver	kidney	muscle	fat
16+	500	7,5 (1,5%)	1,8 (0,36%)	330 (66%)	95 (19%)

	ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+PXL
Meat													
PAH intake cattle (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
ug/l PAH in meat (330kg)	7.3	0.0	3.4	0.0	0.0	0.1	40.5	0.0	0.0	0.6	0.1	0.0	0.1
Human intake (0.3kg meat)	2.2	0.0	1.0	0.0	0.0	0.0	12.2	0.0	0.0	0.2	0.0	0.0	0.0
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Fat													
PAH intake cattle (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
ug/l PAH in fat (95kg)	25.5	0.0	11.9	0.0	0.0	0.3	140.8	0.0	0.0	2.0	0.2	0.1	0.3
Human intake (0.050kg fat)	1.3	0.0	0.6	0.0	0.0	0.0	7.0	0.0	0.0	0.1	0.0	0.0	0.0
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Liver													
PAH intake cattle (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
ug/l PAH in liver (7.5kg)	322.9	0.0	151.3	0.4	0.0	4.2	1782.9	0.1	0.1	25.1	2.7	0.8	3.5
Human intake (0.100kg liver)	16.1	0.0	7.6	0.0	0.0	0.2	89.1	0.0	0.0	1.3	0.1	0.0	0.2
ug/kg bw (60 kg EMEA)	0.3	0.0	0.1	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0

Kidney													
PAH intake cattle (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	2422	0	1135	3	0	32	13372	1	0	188	21	6	27
ug/l PAH in kidney (1.7kg)	1424.7	0.0	667.4	1.9	0.1	18.6	7866.0	0.5	0.2	110.7	12.1	3.4	15.6
Human intake (0.050kg kidney)	71.2	0.0	33.4	0.1	0.0	0.9	393.3	0.0	0.0	5.5	0.6	0.2	0.8
ug/kg bw (60 kg EMEA)	1.2	0.0	0.6	0.0	0.0	0.0	6.6	0.0	0.0	0.1	0.0	0.0	0.0

ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

ANNEX XI Calculated intake VOCs - pig

Calculated carry-over and intake of VOCs via **pigs** using maximum measured concentrations in feed ingredients.
Intake via **meat and organs** of pigs 27 weeks, 110 kg.

Feeding regime	
	kg
Compound feed	2.2
Total	2.2

Percentage of feeding regime	
Category	Percentage
1	25.23
2a	0.02
2b	3.90
5	1.60

Weight of (organs of) pigs					
Age	Weight	organs (kg, %)			
Weeks	kg	Liver	Kidney	Muscle	Fat
27	110	1.5 (1,4%)	0.31 (0.28%)	47 (43%)	38 (35%)

	ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+PXL
Meat													
PAH intake pig (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
ug/l PAH in meat (47kg)	32.3	0.0	14.3	0.2	0.0	2.0	133.5	0.0	0.0	14.1	0.2	0.9	6.1
Human intake (0.3kg vlees)	9.7	0.0	4.3	0.1	0.0	0.6	40.0	0.0	0.0	4.2	0.1	0.3	1.8
ug/kg bw (60 kg EMEA)	0.2	0.0	0.1	0.0	0.0	0.0	0.7	0.0	0.0	0.1	0.0	0.0	0.0
Fat													
PAH intake pig (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
ug/l PAH in fat (38kg)	39.9	0.0	17.7	0.3	0.0	2.5	165.1	0.0	0.0	17.4	0.2	1.2	7.5
Human intake (0.050kg fat)	2.0	0.0	0.9	0.0	0.0	0.1	8.3	0.0	0.0	0.9	0.0	0.1	0.4
ug/kg bw (60 kg EMEA)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Liver													
PAH intake pig (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
ug/l PAH in liver (1.5kg)	1010.7	0.0	449.0	7.5	0.1	62.3	4182.3	0.3	0.1	441.5	6.2	29.5	190.8
Human intake (0.100kg liver)	50.5	0.0	22.5	0.4	0.0	3.1	209.1	0.0	0.0	22.1	0.3	1.5	9.5
ug/kg bw (60 kg EMEA)	0.8	0.0	0.4	0.0	0.0	0.1	3.5	0.0	0.0	0.4	0.0	0.0	0.2
Kidney													
PAH intake pig (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	1516	0	674	11	0	93	6274	0	0	662	9	44	286
ug/l PAH in kidney (0.31kg)	4890.3	0.1	2172.7	36.4	0.3	301.4	20237.1	1.3	0.7	2136.4	29.9	142.6	923.4
Human intake (0.050kg kidney)	244.5	0.0	108.6	1.8	0.0	15.1	1011.9	0.1	0.0	106.8	1.5	7.1	46.2
ug/kg bw (60 kg EMEA)	4.1	0.0	1.8	0.0	0.0	0.3	16.9	0.0	0.0	1.8	0.0	0.1	0.8

ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

ANNEX XII Calculated intake VOCs - chicken

Calculated carry-over and intake of VOCs via **chicken** using maximum measured concentrations in feed ingredients.
Intake via **eggs** of chicken 4 kg, egg production percentage 50%, egg weight 60 grams.

Feeding regime	kg
Compound feed	0.151
Total	0.151

Percentage of feeding regime	
Category	Percentage
1	14.55
2a	0.00
2b	3.24
5	12.01

Feed intake per animal (g/d)	Eggpercentage:		
Body weight (kg)	50%	70%	90%
4	151	163	176

	ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+PXL
Eggs													
PAH intake chicken (in ug)	193	0	29	1	0	5	271	0	0	80	0	2	16
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	193	0	29	1	0	5	271	0	0	80	0	2	16
ug/l PAH in egg (0.030kg)	6440.2	0.0	982.7	19.1	0.0	167.9	9024.1	0.0	0.0	2655.0	11.0	82.0	541.8
Human intake (0.100kg egg)	644.0	0.0	98.3	1.9	0.0	16.8	902.4	0.0	0.0	265.5	1.1	8.2	54.2
ug/kg bw (60 kg EMEA)	10.7	0.0	1.6	0.0	0.0	0.3	15.0	0.0	0.0	4.4	0.0	0.1	0.9

ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.

Intake via **meat and organs** of chicken 42 days, 2500 grams.

Feeding regime	kg
Compound feed	0.16
Total	0.16

Percentage of feeding regime	
Category	Percentage
1	26.60
2a	3.40
2b	4.00
5	2.30

Weight of (organs of) chicken					
Age	Weight	Organs (g, %)			
Days	g	Liver	Kidney	Muscle	Fat
42	2500	53 (2.1%)	20 (0.8%)	1500 (60%)	
	2800	59 (2.1%)	23 (0.8%)	1680 (60%)	

	ACT	BNZ	CHX	EAC	EBZ	HEP	HEX	HXE	OCT	PEN	TOL	OXL	M+PXL
Meat													
PAH intake chicken (in ug)	158	0	118	16	1	36	712	5	3	118	12	16	36
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	158	0	118	16	1	36	712	5	3	118	12	16	36
ug/l PAH in meat (1.5kg)	105.2	0.3	78.9	10.7	0.9	24.0	474.8	3.3	1.7	78.6	8.2	10.9	24.3
Human intake (0.3kg meat)	31.6	0.1	23.7	3.2	0.3	7.2	142.4	1.0	0.5	23.6	2.5	3.3	7.3
ug/kg bw (60 kg EMEA)	0.5	0.0	0.4	0.1	0.0	0.1	2.4	0.0	0.0	0.4	0.0	0.1	0.1
Liver													
PAH intake chicken (in ug)	158	0	118	16	1	36	712	5	3	118	12	16	36
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	158	0	118	16	1	36	712	5	3	118	12	16	36
ug/l PAH in liver (0.053kg)	2977.5	8.0	2233.6	301.5	24.5	678.6	13438.1	94.4	47.2	2223.7	231.8	307.1	686.6
Human intake (0.100kg liver)	148.9	0.4	111.7	15.1	1.2	33.9	671.9	4.7	2.4	111.2	11.6	15.4	34.3
ug/kg bw (60 kg EMEA)	2.5	0.0	1.9	0.3	0.0	0.6	11.2	0.1	0.0	1.9	0.2	0.3	0.6
Kidney													
PAH intake pig (in ug)	158	0	118	16	1	36	712	5	3	118	12	16	36
Used transfer in %	100	100	100	100	100	100	100	100	100	100	100	100	100
PAHs transferred (in ug)	158	0	118	16	1	36	712	5	3	118	12	16	36
ug/l PAH in kidney (0.020kg)	7890.4	21.2	5919.0	799.0	65.0	1798.3	35610.9	250.2	125.1	5892.8	614.3	813.8	1819.5
Human intake (0.010kg kidney)	78.9	0.2	59.2	8.0	0.7	18.0	356.1	2.5	1.3	58.9	6.1	8.1	18.2
ug/kg bw (60 kg EMEA)	1.3	0.0	1.0	0.1	0.0	0.3	5.9	0.0	0.0	1.0	0.1	0.1	0.3

ACT=Acetone, BNZ=Benzene, CHX=Cyclohexane, EAC=Ethylacetate, EBZ=Ethylbenzene, HEP=Heptane, HEX=Hexane, HXE=1-Hexene, OCT=Octane, PEN=Pentane, TOL=Toluene, OXL=O-Xylene, M+PXL=M+P Xylene.