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Cancer risk assessment of azo dyes and aromatic amines from garment and footwear

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Samenvatting

Dit rapport beschrijft een schatting voor het risico op kanker dat verbonden is aan het dragen van kleding en schoeisel waarin kankerverwekkende azo kleurstoffen aangetroffen zijn. In de risicoschatting wordt de (geschatte) hoeveelheid aromatische aminen die tijdens het dragen van kleding en schoeisel in het lichaam opgenomen kan worden vergeleken met het chronische, acceptabel geachte, blootstellingsniveau van deze aminen. In de risicoschatting wordt met de volgende aspecten rekening gehouden: *1)* de kans dat azo kleurstoffen en aminen in kleding en schoeisel voorkomen, *2)* het aantal malen dat kleding en schoeisel gedragen wordt, *3)* de mate waarin kleding en schoeisel met de huid in contact komen, *4)* de hoeveelheid aminen en azo kleurstoffen die in kleding en schoeisel voorkomen, *5)* de mate waarin azo kleurstoffen en aminen met zweet uit kledingtextiel en schoeisel uitlogen, *6)* de opname door de huid van azo kleurstoffen en aminen en *7)* de acceptabele limieten zoals die gesteld zijn voor de chronische bloostelling aan kankerverwekkende aromatische aminen. Deze laatste limieten zijn gesteld op de blootstellingsniveaus die leiden tot 1 extra geval van kanker per miljoen levenslang blootgestelden ("Negligible Risk Level", NRL) resp. 1 extra geval van kanker per tienduizend levenslang blootgestelden ("Maximum Permissible Risk Level", MPRL).

Het risico op kanker is voor 6 stuks kledingtextiel, 2 stuks textiel schoeisel en 2 stuks leren schoeisel berekend. In deze producten zijn benzidine, *o*-tolidine, *o*-dianisidine en 2,4-toluenediamine aangetroffen. In alle gevallen bleek het dragen van de onderzochte producten te leiden tot een blootstelling aan aminen die ligt boven de NRL. In 3 onderzochte stuks kledingtextiel bleek deze blootstelling zelfs boven de MPRL te liggen.

Bij genoemde resultaten moet het volgende worden opgemerkt. Azo-kleurstoffen ontlenen hun carcinogene werking aan een in het kleurstofmolecuul aanwezig aromatisch amine. Het gezondheidskundige risico van producten waarin azo-kleurstoffen voorkomen wordt dan ook bepaald door de hoeveelheid van deze aminen die bij gebruik van het product in het lichaam terecht kunnen komen. In dit rapport is voor alle onderzochte aromatische aminen dezelfde risicogrens gebruikt in de risicoschattingen. Deze grens ($1x10^{-6}$ bij 0,3 ng amine per persoon per dag) is door het "U.S. Environmental Protection Agency" (U.S. EPA) afgeleid uit epidemiologische gegevens voor de blootstelling van werknemers aan benzidine. Een alternatieve benadering is de afleiding van risicogrenzen uit carcinogeniteitstudies met aromatische amines in proefdieren. Deze benadering, die recent voor verschillende aminen is toegepast door de Commissie WGD van de Gezondheidraad, leidt tot risicogrenzen die aanzienlijk hoger zijn dan die welke uit epidemiologisch onderzoek afgeleid is, en dus tot een aanzienlijk lager risico. Wanneer deze alternatieve benadering toegepast zou zijn in de in dit rapport gepresenteerde risicoschattingen dan zou het risico op kanker in slechts één van de onderzochte gevallen boven het Verwaarloosbare Risiconiveau komen te liggen. Er zou in geen van de schattingen nog sprake zijn van overschrijding van het Maximaal Toelaatbare Niveau.

Summary

This report describes a quantitative cancer risk assessment for individuals wearing garment and footwear which are coloured with azo dyes.

Basically the risk assessment consists of a comparison of the (estimated) amount of aromatic amines which, during the wearing of garment and footwear, enters the body and the acceptable limits as set for the chronic exposure to these amines. In the risk assessment the following aspects are taken into account: *1)* the chance that garment and footwear contain azo dyes and amines, *2)* the frequency with which garment and footwear is worne, *3)* the fraction of garment and footwear which comes into contact with the skin, *4)* the amount of amines in garment and footwear, *5)* the leaching of azo dyes and amines from garment and footwear with sweat, *6)* the absorption of azo dyes and amines through the skin and *7)* the acceptable limits as set for the chronic exposure to aromatic amines. For the latter limits the "Negligible Risk Level" (NRL), i.e. the life-long exposure which leads to 1 extra case of cancer in one million exposed persons, and the "Maximal Permissible Risk Level" (MPRL), i.e. the lifelong exposure which leads to 1 extra case of cancer in ten thousend exposed persons, were taken.

A quantitative risk assessment was performed for six pieces of garment, two pieces of textile footwear and two pieces of leather footwear. In these products benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenedimine were detected. In all cases the wearing of these products leads to an exposure of amines exceeding the NRL. In three pieces of garment the calculated exposure even exceeded the MPRL.

In interpreting the results mentioned above the following should be kept in mind. Azo-dyes exert their carcinogenic action by means of an aromatic amine. This amine is part of the dye. The cancer risk of consumer products which contain azo-dyes thus is determined by the amount of the amine which, when using the product, enters the body. In this report the same carcinogenic risk level is used for all of the amines investigated, i.e. an exposure of 0.3 ng amine per person per day was assumed to be associated with a $1x10^{-6}$ " life-long" cancer risk. This risk level was obtained by "U.S. Environmental Protection Agency" (U.S. EPA) from epidemiological data on the exposure of workers to benzidine. Alternativily the carcinogenic risk level of aromatic amines may be obtained from studies in experimental animals. This approach, which has recently been taken by the Dutch Expert Committee on Occupational Standards (DECOS) of the Health Council of the Netherlands, leads to risk levels which are considerably higher than the one which was deduced by U.S. EPA from epidemiological data. Hence, substantially lower risks are calculated with the method used by DECOS. With this method in only one of the investigated cases would the wearing of the product lead to a cancer risk above the NRL. In none of the cases would the risk be above the MPRL.

1. Introduction

1.1 Historical background

In 1995 the results of a study by the Regional Inspectorate for Health Protection (in dutch: *Keuringsdienst van Waren, Inspectie Gezondheidsbescherming Alkmaar*) showed the presence of azo dyes in a number of the analysed samples of garment and other textiles. The azo dyes contained benzidine and/or other aromatic amines such as *o*-tolidine as biologically active compounds (Van Haperen and Hiemstra, 1995). Benzidine and a large number of other aromatic amines have been classified as human or animal carcinogen, amongst others by the World Health Organisation (International Agency for Research on Cancer, IARC) and the European Union (EU)[1]. The presence of azo dyes in textiles can result in dermal exposure (by the wearing of garment) and, especially in children, in oral exposure (by sucking on clothing and textile toys).

In the above mentioned study the total concentration of the azo dye was determined in the samples. However, as the actual exposure of consumers depends on the degree of leaching of the dye with sweat or saliva from articles and the contact with garment, this measure cannot be used for risk assessment purposes. For that reason, the Regional Inspectorate for Health Protection conducted an additional study, in which the leaching with sweat of azo dyes and their constituing amines was simulated in a set of underwear in which a benzidine-based azo dye had been detected. The results of this study show that only a minor part of the total amount of the azo dye present in the underwear may be extracted with sweat and, hence, may be available for exposure (Van Haperen, personal communication).

A risk assessment by the National Institute of Public Health and the Environment (RIVM), departing for the extractable amount of azo dye or "free" benzidine, instead of the total amount of these compounds in the product, showed that the acceptable risk level with respect to carcinogenicity may still be exceeded by wearing underwear coloured with the aforementioned benzidine-based azo dye (Speijers *et al.*, 1996). Based on these findings the Ministry of Welfare, Health and Sports introduced a ban on the trade in clothing, footwear and bed-clothes coloured with azo dyes from which carcinogenic amines can be formed (Commodity Act Regulation Azo-dyes: Official State Journal (in dutch: *Staatscourant* 143, 29 July 1996). The regulation includes all azo dyes based on one of twenty primary amines specified in the regulation. These twenty amines are the same as those banned in German legislation on consumer products intended for long-term contact with the skin, including clothing and towels (Consumer Goods Ordinance, cited in Mensink *et al.*, 1997). In April 1998 the Commodity Act Regulation was implemented into the Commodity Act *Besluit* (*Staatsblad* 339, 1998). This *Besluit* does not apply to pigments and, until January 2000, not to second-hand articles, articles made of recycled fibres, and articles (clothing and footwear) used for personal protection.

[1] In the 22nd amendment of EU Directive 67/548/EEC, September 1996, azo dyes which are based on benzidine, were also classified as carcinogen.

In the Netherlands and other European countries, there are some additional national or EU regulations that limit or prohibed the use of individual carcinogenic amines (especially benzidine, 2-naphtylamine and 4-aminobiphenyl which have been classified as human carcinogens) and azo dyes in consumer products, viz. preparations (such as shoe polish), foodstuffs, food packaging and cosmetics. There is, however, no general ban on the production, trade or use of these substances (Mensink *et al.*, 1997). A survey by the Netherlands Inspectorate of Environmental Hygiene carried out in 1994/1995 revealed that azo dyes not only enter the Netherlands in the products that are regulated in Commodities Act, but that a number of these dyes themselves are imported, applied and traded in the Netherlands (Klingenberg *et al.*, 1997 as cited in Mensink *et al.*, 1997). This survey did not show, however, which azo dyes and which quantities thereof are used in the Netherlands, nor in which consumer products azo dyes may be present. For this reason, the Ministry of Housing, Spacial Planning and the Environment ordered a broad survey on the presence of azo dyes in consumer products (with special regard to products which are not regulated in the Commodity Act) and on the regulations on azo dyes in other countries. In this survey, conducted in 1997 by Consultancy and Research for Environmental Management (CREM), a screening of relevant product-dye combinations was made from the data on possible applications, actual concentrations in products (using a large date base of test results from the Netherlands and Germany), potential exposure (degree of contact) and the existing regulatory legislation. The results of this survey (Mensink *et al.* 1997) show that, in addition to clothing and footwear, other textile and leather products such as textile toys, household textiles, carpets and leather watch straps may need further attention, as well as a number of products of other materials, including paper products (diapers, origami and other decorative/hobby paper), cosmetics, shoe polish, and ink and paints (ecoline or textile paints).

1.2 Cancer risk assessment of garment and footwear

This report presents a cancer risk assessment for the dermal (and in one case the oral) exposure of consumers to garment and footwear. The product groups garment and footwear were selected on the available "positive" samples in which the leaching of azo dyes and amines with a sweat simulant could experimentally be determined [2]. The amines detected in garment and footwear in the Netherlands included benzidine (CAS No. 92-87-5), *o*-tolidine (3,3'-dimethylbenzidine: CAS No. 119-93-7), *o*-dianisidine (3,3'-dimethoxybenzidine: CAS No. 119-90-4)) and 2,4-toluenediamine (CAS No. 95-80-7). The data of the 1996 risk assessment for the wearing of underwear (Speijers *et al.*, 1996) are included in this report, but the exposure assessment has been adapted in conformity with the currently used method.

In 1999 part two of the risk assessment will be conducted. In that part of the study the risk of exposure by the use of other products, to be selected on the basis of the above mentioned survey by CREM and additional data on exposure profiles, will be assessed.

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[2]By far most available data on azo dye concentrations in consumer products are of textile and leather. The reason for this is that, in particular, products which are made of these materials are included in the major Dutch and German regulations for azo dyes in consumer products. Consequently, products which consist of these materials are frequently monitored on the presence of azo dyes.

2. Exposure Assessment

2.1 Principles of exposure assessment

The risk assessment of azo dyes in garment and footwear has two components. Firstly, azo dyes may leach with sweat or saliva from a product onto the skin. Once on the skin the dye may be absorbed (Aldrich *et al.*, 1986; Collier *et al.*, 1993) or, in the case of oral exposure, be swallowed. In the skin part of the absorbed azo dye can be split into its consituting amine (Collier *et al.*, 1993). The amine may then migrate to the blood. Splitting of the dye may also occur in the liver and in the gastro-intestinal tract (Chung *et al.*,1983). The released amine may enter the body by absorption across the gastro-intestinal wall (Lynn *et al.*, 1984). Furthermore, when present in the product, the amine itself ("free" amine) may leach onto the skin. As with amine which is released from an azo dye in the skin or the gastro-intestinal tract the leached "free" amine is taken up by absorption through the skin or by absorption across the wall of the gastro-intestinal tract.

2.2 Assessing the uptake of carcinogenic amines from consumer products

In the current "EU Technical Guidance Documents for the risk assessment of new and existing substances" a method is described for the estimation of the uptake of chemicals from consumer products (see Fig. 1). This method has recently been used for a risk assessment of azo dyes from textiles (LGC, 1998, by order of the European Committee). In this method the contribution of "free" amines to the risk of azo dyes is not taken into account. For the dermal route of exposure this method is as follows (see Fig. 1). Given the (potential) amount of the azo dye which can migrate from the product ($A_{derm,pot}$, g) and assumptions on the rate at which azo dyes migrate from the product onto the skin, the duration of the exposure, the human body weight (bw) and the absorption of the azo dye through the skin the daily uptake of the dye, i.e. $E_{eff,dye}$ (g/kg bw/day), is calculated. Next an assumption is made on the conversion of the dye into its carcinogenic amine and the uptake of the amine in the body, i.e. $E_{eff,amine}$ (g/kg bw/day).

In its 1996 report on the risk assessment of benzidine containing garment RIVM used a different approach to assess the uptake of the amine benzidine from (two pieces of) underwear. In the 1996 report the uptake of benzidine was calculated as the sum of the uptake of "free" benzidine and benzidine which can be split off from an azo dye. As such this calculation was based on the amount of azo dye and "free" amine which may leach from the product with a sweat simulant. Other entities included on the calculation were the effect of washing on the leaching of azo dyes from textile, the frequency with which exposure takes place, the conversion of the leached dye into benzidine and the uptake of benzidine in the body (see Speijers *et al.*, 1996 for further details).

After the appearance of the 1996 RIVM report new data on the effect of washing on the leaching of azo dyes from textile have become available (ETAD, 1997). Furthermore, RIVM's 1996 risk assessment was limited to one specimen of one category of products, i.e. one piece of underwear, in which the total product comes into contact with the skin and it was

limited to benzidine. The need to include more products necessitated an update of RIVM's 1996 approach to assess the uptake of carcinogenic aromatic amines. A detailed description of the information used in the update for garment and footwear and the references on which this update is based is given in Appendices 1 and 2. Fig. 2 summarizes the concept of this update. In short the approach proposed is as follows. Exposure assessment starts with an estimation of the probability that consumers buy a product which contains carcinogenic azo dyes and amines[3]. For such a product the amounts of "free" amine and amine containing azo dye which may leach with sweat are experimentally determined. Next the number of times that consumers come into contact with the product during its life cycle[4] (contact frequency) and the part of the product which comes into contact with the skin (skin contact factor) are taken into account. Applying these factors on the leached amounts of azo dye and "free" amine results in the calculation of the amounts of azo dye ($A^*_{derm,dye}$, g) and "free" amine ($A^*_{dermal,"free" amine}$, g) which may reach the skin. After correction for the absorption of the azo dye and the conversion of the absorbed dye into its constituting amine the total uptake of the amine ($E_{eff,total\ amine}$, g/day) is calculated as the sum of the absorbed amounts of "free" amine and amine which is released from the azo dye.

To perform the calculation depicted in Fig. 2 the leaching of azo dyes and amines with a sweat/saliva needs to be known. Unfortunately this information is not available for either of the products which have been mentioned in the CREM survey. Therefore a selection of available product items was made for analysis of leaching[5]. This selection was made on the basis of detected amounts of azo dyes (highest amount, highest risk) and evaluation of the carcinogenic potency of the detected amines in experimental animals (see paragraph 3.2.2).

Although a larger diversity of products was aimed at only five pieces of clothing and four pieces of footwear appeared to be available for experimental research. For each of these products the leaching with sweat simulant of "free" amines and amines which are part of an azo dye (benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine) was determined by the Regional Inspectorate for Health Protection. The results of this investigation were combined with the results of RIVM's previous investigation on the leaching of benzidine and a benzidine containing azo dye from underwear (Speijers *et al.*, 1996).

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[3]When one is only interested in the cancer risk which can be attributed to the product as such the mentioned chance has to be left out of the calculations. In this case the cancer risks presented in Tables 5-8 have to be multiplied by a factor of 12.5 (1.0/0.08, Tables 5 and 6) and 10 (1/0.1, Tables 7 and 8).

[4] life cycle defined as the period between the purchase and the disposal of the product

[5] used data bases: TÜV Rheinland: 40 different products, including textiles like clothing, tea towels, etc., cosmetics, leather products, footwear, carpets as cited in Mensink *et al.*, 1997; TNO: 14 products including textile clothing, textile toys and leather products, as cited in Mensink *et al.*, 1997 and the *Keuringsdienst van Waren*: textile clothing, textile toys and paper work (Van Haperen, 1997; Laurensse, 1998, Van Vliet, 1998)

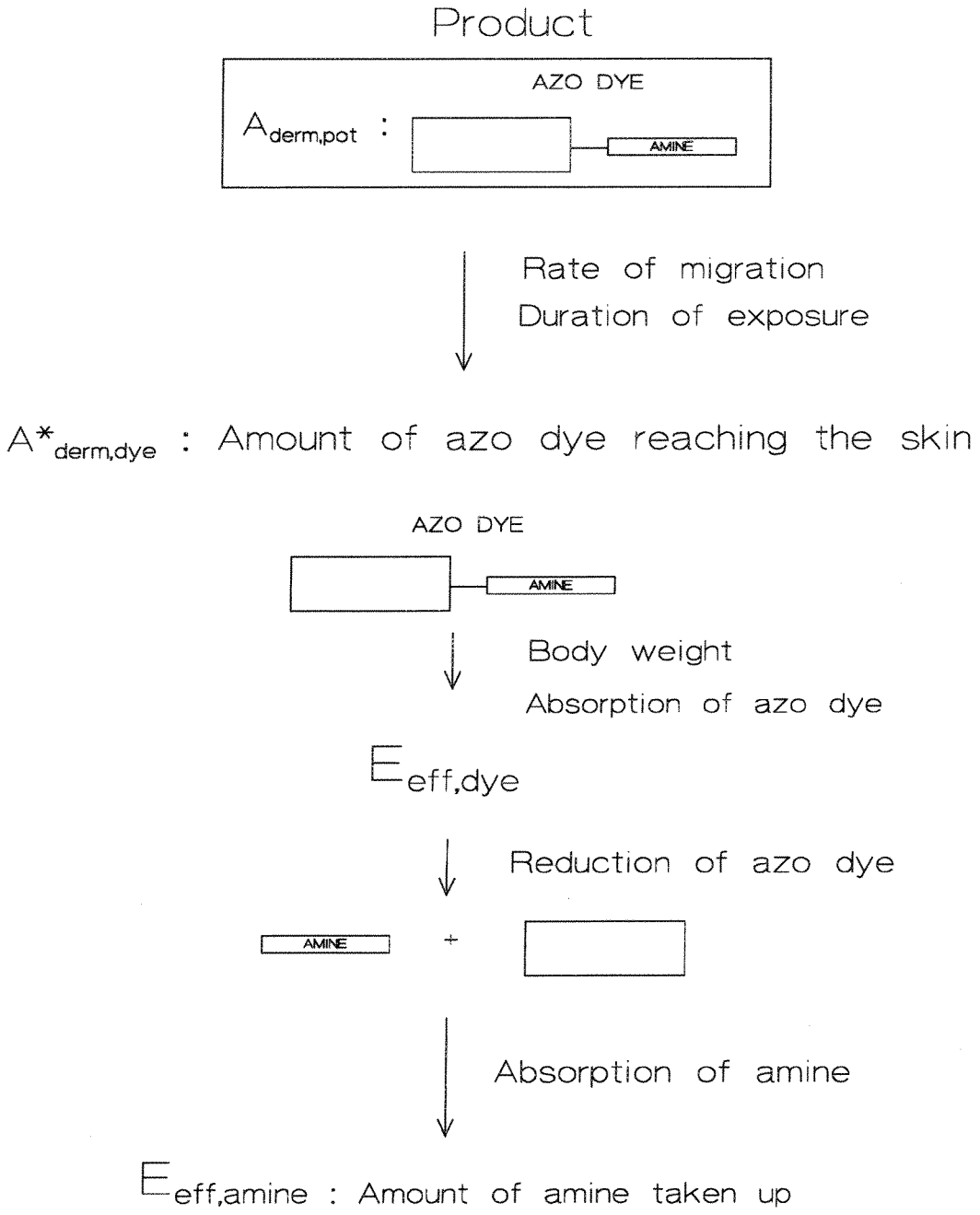

Fig. 1 Estimation of the dermal uptake of carcinogenic amines from products containing azo dyes (Appendix IV in EC, 1996).

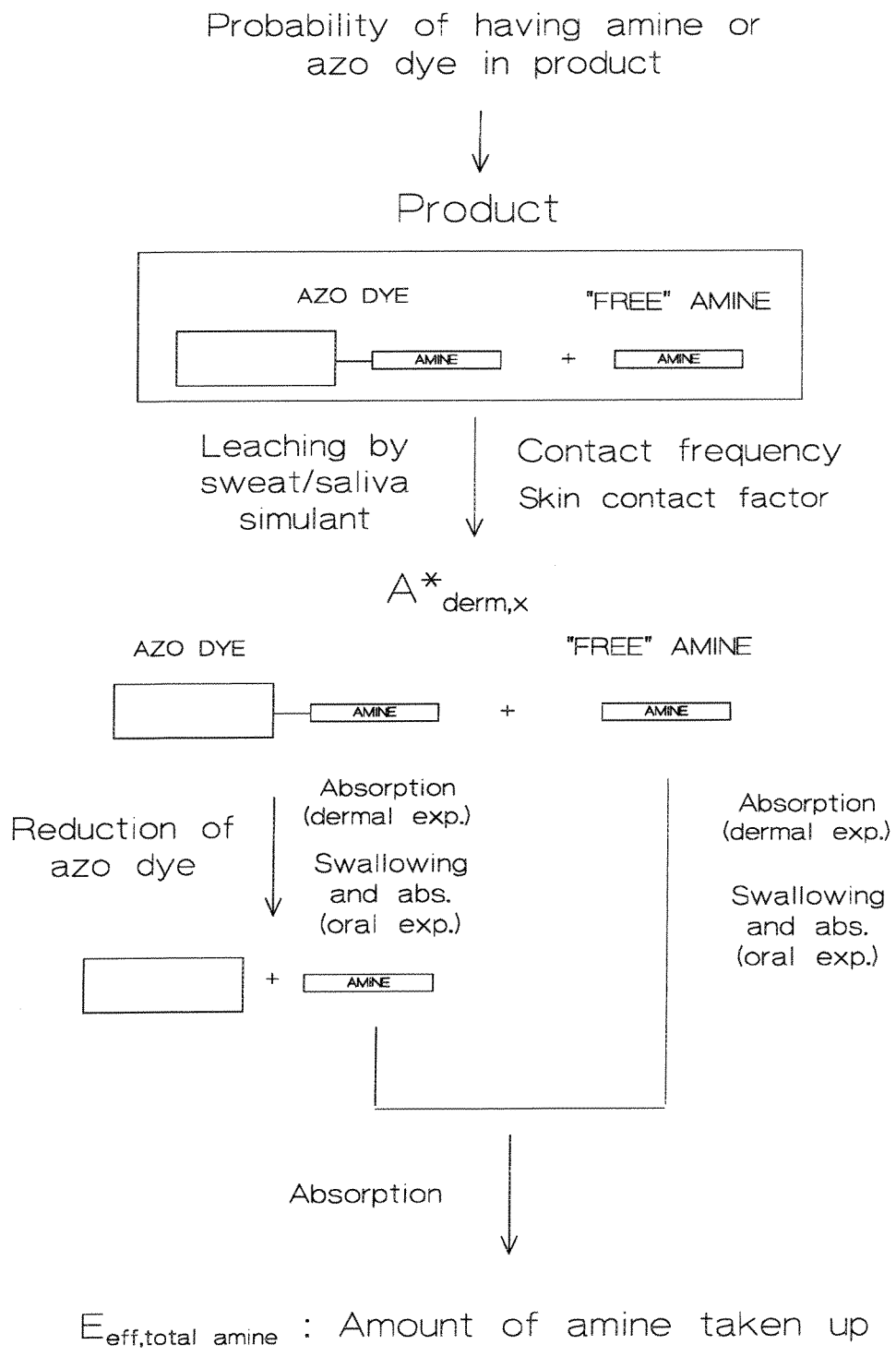

Fig. 2 Estimation of the dermal/oral uptake of carcinogenic amines from garment and footwear containing azo dyes and "free" amines. x: azo dye or "free" amine (This report).

The experimentally determined amount of leached amine was used as the starting points for the calculation of $E_{eff,total\ amine}$.[6] This calculation, which is presented in detail in Tables 1-4 of Appendix 1, is represented by the following equation:

$$E_{eff,total\ amine} = P \times F_{cont.} \times A_{amine} \times W \times F_{skin} \times F_{abs}$$

with:

$E_{eff,total\ amine}$	Expected daily human uptake of amine (sum of "free" amine and amine which is part of an azo dye) (g/day)
P	Probability that a product contains a carcinogenic azo dye, cq. carcinogenic amine (no dimension).
$F_{cont.}$	Frequency of contact with the product (day^{-1})
A_{amine}	The amount of amine which leaches with sweat simulant from one gram of a product (sum of the leached amount of "free" amine and the amount of amine which leaches as part of an azo dye, g/g)[7]
W	Weight of dye/amine containing material (g)
F_{skin}	Skin contact factor (no dimension)
F_{abs}	Fraction absorbed (no dimension).

In calculating $E_{eff,total\ amine}$ no correction for "peak vs. repeated exposure" was made (see Appendix 1).

The calculated daily uptake of the amines are presented in tables 1-4. A comparison of the uptakes in Table 1 and in Table 3 with those in Table 2 and Table 4 shows that the major part of the uptake of amines from garment and footwear is caused by amines which leach as part of an azo dye, and not by leaching of the "free" amine.

[6] A recent report of the National Institute of Occupational Health Denmark (VKI, 1998) also used the amount of azo dyes which leaches with a sweat simulant as the basis for the risk assessment of consumer exposure to garment. In this study aromatic amines were detected in 33 out of 59 investigated pieces.

[7] note that by substituting only the amount of "free" amines which leaches from a product into the equation the expected daily human uptake of the "free" amine, $E_{eff,"free"amine}$, is obtained.

Table 1. Calculated daily uptake of aromatic amines resulting from consumer exposure to azo dyes in garment ("free" aromatic amines included)

Product\ Material\ Route of exposure	Amine*	Amount# (µg/g)	Leachable amount & (µg/g) (% leached)	$E_{eff,total\ amine}$ (ng/day)
Underwear unknown\dermal	benzidine	1368	0.63 (0.05)	7.5
Blouse silk\dermal	*o*-tolidine	2796	3.8 (0.14)	119
Children's legging cotton-elastine dermal	benzidine	1112	1.8 (0.16)	59
String of children's sweater cotton\oral	*o*-dianisidine	337	0.49 (0.15)	6.4
Underwear silk\dermal	benzidine	3138	5.7 (0.18)	285
Lining of a children's coat polyamide\dermal	*o*-dianisidine	110	0.50 (0.45)	1.0
	o-tolidine	35	0.18 (0.51)	0.36
	benzidine	17	0.63 (3.7)	1.3

* Detected amine
# Total amine, i.e. the sum of the amounts of "free" amine and amine as part of an azo dye
& Total leachable amount, i.e. the sum of the amounts of "free"amine and amine as part of an azo dye which leaches from the product with a sweat simulant

Table 2. Calculated daily uptake of aromatic amines resulting from consumer exposure to "free" aromatic amines in garment

Product\ Material\ Route of exposure	Amine*	Amount (µg/g)#	Leachable amount (µg/g)&	$E_{eff,"free" amine}$ (ng/day)
Underwear	benzidine	unknown	0.09	1.8
Blouse	o-tolidine	unknown	< 0.05	< 1.6
Children's legging	benzidine	unknown	0.055	1.8
String of children's sweater	o-dianisidine	unknown	< 0.05	< 0.07
Underwear	benzidine	unknown	0.16	8.0
Lining of a children's coat	o-dianisidine	unknown	< 0.05	< 0.1
	o-tolidine	unknown	< 0.05	< 0.1
	benzidine	unknown	< 0.05	< 0.1

* as in Table 1# Amount of "free" amine & Amount of "free" amine which leaches with sweat simulant

Table 3. Calculated daily uptake of aromatic amines resulting from consumer exposure to azo dyes in footwear ("free" aromatic amines included)

Product\ Material\ Route of exposure	Amine*	Amount (µg/g)#	Leachable amount (µg/g)& (% leached)	$E_{eff,total amine}$ (ng/day)
Children's slipper textile\dermal	benzidine	381	3.8 (1.0)	3.0
Children's slipper textile\dermal	2,4-toluene diamine	210	0.17 (0.09)	0.41
Shoe (upper side) leather\dermal	benzidine	161	24 (14.9)	13.2
Sportsshoe (upper side) leather\dermal	*o*-dianisidine	589	231 (39)	4.8

* as in Table 1 # as in Table 1& as in Table 1

Table 4. Calculated daily uptake of aromatic amines resulting from consumer exposure to "free" aromatic amines in footwear

Product\ Material\ Route of exposure	Amine*	Amount (µg/g)#	Leachable amount (µg/g)&	$E_{eff,"free" amine}$ (ng/day)
Children's slipper textile\dermal	benzidine	unknown	0.06	0.0049-0.049
Children's slipper textile\dermal	2,4-toluenediamine	unknown	< 0.1	< 0.076
Shoe (upper side) leather\dermal	benzidine	unknown	0.22	0.18-1.8
Sportsshoe (upper side) leather\dermal	*o*-dianisidine	unknown	0.35	0.03-0.3

* as in Table 2 # as in Table 2& as in Table 2

3. Effect Assessment

3.1 Hazard identification

Epidemiological studies have provided evidence for at least some azo-dye derived aromatic amines as being human carcinogens: i.e. benzidine and 2-naphthylamine were shown to induce urinary bladder cancer in workers in azo-dye industry (see IARC, 1974; 1982; 1987, and references cited therein). Additionally, most aromatic amines tested in experimental animals also appear to be carcinogens. Target-sites for carcinogenesis in animals depend on the route of administration of the amine, and include liver, urinary bladder, hemopoietic organs, mammary gland, zymbal gland, and gastro-intestinal tract (Gold and Zeiger, 1997). Though many aromatic amines have target-sites in common, no clear relationship can be established between the structural features of an amine and its target organs.

3.2 Dose-response assessment

From the preceeding chapter on exposure it is clear that, for the purpose of this report, the dose-response analysis can be restricted to benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine, i.e. amines found in garment and/or footwear at detectable levels. All four amines were shown to be carcinogenic in experimental animals (Gold and Zeiger, 1997). Preferentially, however, dose-response analysis should be based on observations in humans instead of on animal data. Of the four amines considered, only the carcinogenicity of benzidine has been evaluated in humans (see next section). For this reason benzidine is the amine of choice, i.e. the amine to which the carcinogenic potency of other aromatic amines has to be referred to, if possible.

3.2.1 Benzidine

Although benzidine clearly is established as a human carcinogen in certain occupational settings, accurate concurrent exposure assessments usually are not performed in these studies. This hampers a direct quantitative assessment of the dose-response relationship between benzidine exposure and bladder tumour induction in the exposed humans. Besides, because of their proven carcinogenic activity towards humans, and the fact that benzidine and its associated azo-dyes are not produced in the Netherlands[8], a limit value, therefore, has not been established, neither for the general population nor for workers.

A quantitative risk assessment on benzidine that can be used in this report, is the one described by the U.S.EPA in 1993 (U.S. EPA, 1997). This limit value has also been used by others in their assessment of cancer risks associated with the wearing of textiles and leather goods coloured with azo dyes, e.g. LGC (LGC, 1998). In their assessment U.S. EPA has estimated benzidine exposure in one of the industrial cohort studie described by Zavon *et al.* (1973), in an indirect way. In this assessment two types of observations were used: the

[8] Azo dyes are not produced in the Netherlands and the use is limited to the colouring of mineral oil products, primarily diesel oil, which is coloured with solvent red dyes (Mensink *et al.*, 1997).

benzidine levels in urine of exposed workers in this cohort and the relationship between oral benzidine exposure and urinary excretion determined in rhesus monkeys (Rinde and Troll, 1975). Based upon these data an oral slope factor of 230 per mg benzidine per kg body weight per day was calculated (U.S. EPA, 1997). This factor was derived by incorporating the usual assumptions and correction factors, and by applying a one-hit extrapolation model. From this a lifetime daily exposure corresponding to a risk of one per million can be calculated (via linear extrapolation) to be 0.3 ng benzidine per person per day (for details see Appendix 2.1). A comparable figure of 0.48 ng a day is obtained when these data used by U.S.EPA are combined with methodology adopted in the Netherlands (HCN, 1994a; for details see Appendix 2.1).

Clearly, this U.S.EPA approach has some specific uncertainties that will be further discussed in paragraph 5.2.

3.2.2 Other aromatic amines

In contrast with benzidine, carcinogenicity data in humans are not available for the amines *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine. The most straightforward way to relate the human carcinogenicity of these amines to that of benzidine is to mutually compare their dose-response characteristics in experimental animals. This approach seems justified by their clear structural similarity and anticipated pathways of bioactivation (i.e. oxydation and acetylation of the critical aromatic amine group; Chou *et al.*, 1995). Animal bioassay data are available for all four mentioned amines in the same species, i.e. the rat (though exposure to benzidine was by inhalation, whereas the other amines were administered orally). Interestingly, all compounds have some overlap with respect to their target organs for carcinogenesis, i.e. the liver, mammary gland, and the hemoreticular system (see previous paragraph, also see Gold and Zeiger, 1997). The most convenient way of comparing the relative carcinogenic potency of these compounds is by using a similar potency estimate, e.g. the TD_{50} as developed by Gold and Zeiger (1997; see Appendix 2.2). This TD_{50} is defined as the dose rate in mg per kg body weight per day required to halve the probability of an experimental animal of remaining tumour free by the end of the standard life-span for the species (Peto *et al.*, 1984, Sawyer *et al.*, 1984). The TD_{50}'s of benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine clearly do not differ significantly from each other (in mg/kg/day): 1.7 (benzidine), 0.3 (*o*-tolidine), 0.6 (*o*-dianisidine), and 2 to 4 (2,4-toluenediamine), respectively.

The equipotency of the above mentioned amines in experimental animals, possibly, may not hold in humans. However, we do not have any information sofar that conflicts with this position. As far as data are available at least some correlation exists between the carcinogenic potency of chemicals in animals and humans (Allen *et al.*, 1988; Dedrick and Morrison, 1992). This would suggest a more or less equal carcinogenic potency of aromatic amines in humans as well. On the other hand, various concentration-dependent mechanisms may underly tumour-induction in animals at high dose levels, which may not contribute to tumour formation at the relatively low exposures that humans experience, and this may vary from compound to compound. Therefore, substantial - though unpredictable - differences in potency may prevail at these lower exposure levels.

3.2.3 Conclusion

This dose-response analysis has been restricted to benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine, as only these amines were found in garment and/or footwear at detectable levels (see previous chapter). Preferentially, dose-response analysis should be based on observations in humans instead of on animal data. Only for benzidine human data on carcinogenicity are available. From these data U.S. EPA arrived at an acceptable 10^{-6} risk levels for benzidine of 0.3 ng per person per day (U.S.EPA, 1997). When methodology in use by the Health Council of the Netherlands is applied to these data a similar figure of 0.48 ng per person per day is obtained.

The dose-response characteristics of benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine in experimental animals suggest equipotency of these compounds with respect to carcinogenicity. Therefore, the toxic equivalency dose of 0.3 ng aromatic amine per person a day, that may be taken to be associated with an additional one per million lifetime cancer risk, applies to all considered amines, i.e. also to *o*-tolidine, *o*-dianisidine, and 2,4-toluenediamine.

It should be realized, though, that this value has a rather high degree of uncertainty. In addition to the points raised above (paragraph 3.2.2), which apply to all risk assessments for genotoxic carcinogens, some uncertainties specific to this benzidine case will be addressed in paragraph 5.2.

4. Risk Assessment

4.1 Acceptable exposure limits for carcinogenic aromatic amines

For carcinogens without a threshold value a 1×10^{-6} lifetime risk level, i.e. one extra case of cancer per million persons exposed lifetime, is considered to be the "negligible risk level" (NRL). A risk level that is 100 times higher (1×10^{-4} lifetime, i.e. one extra case of cancer per 10.000 persons exposed during life-time) is considered to be the "maximum permissible risk level" (MPRL).

4.2 Calculation of cancer risk

Given the calculation of the uptake of a carcinogenic amine from a product ($E_{eff,amine}$, see section 2) and its carcinogenic potency (NRL, being 0.3 ng/person/day for benzidine, *o*-tolidine, *o*-dianisidine and 2,4-toluenediamine, see section 3) the 10^{-6} cancer risk is calculated as the ratio of $E_{eff,total\ amine}$ and the *NRL*.

4.3 Cancer risk: Garment

In the Netherlands the amines benzidine, *o*-tolidine and *o*-dianisidine have been found in garment (Van Haperen, 1997). The cancer risks which correspond with the exposure to these amines are presented in Tables 5 and 6. Table 5 shows calculated cancer risks for the total amount of amine which leaches with sweat simulant, i.e. the sum of "free" amine and amine which is part of an azo dye. Table 6 shows calculated cancer risks for the "free" amine only.

When the total amount of leachable amine is taken into account Table 5 shows that the exposure to all 6 products leads to a cancer risk which is above the NRL. In 3 of these cases the calculated cancer risk even exceeds the MPRL, with the highest risk , i.e. 950×10^{-6} , being calculated for underwear. When only the amount of "free" amine is taken into account Table 6 shows that the exposure to 3, and possibly 4, products leads to a cancer risk above the NRL, but below the MPRL.

Comparison with previous risk assessment

As already mentioned a previous RIVM risk assessment focused on two pieces of underwear containing benzidine and a benzidine containing azo dye (Speijers *et al.*, 1996). In one of these products the risk assessment was based on the total amount of benzidine (calculated range of uptake: 1.8-90 ng per person per day, corresponding cancer risk: 6×10^{-6} - 270×10^{-6}). In the other product only "free" benzidine was taken into account (calculated range of uptake: 0.74-37 ng per person per day, corresponding cancer risk: 2.5×10^{-6} - 120×10^{-6}). When these calculations were repeated according with the scheme depicted in Fig. 2 uptakes of 7.5 and 1.8 ng/person/day (see Appendix 1, Table 1) and cancer risks of 25×10^{-6} and 6×10^{-6} were found. These cancer risks lie at the lower bound of the range of the risk level which has previously been calculated for these products.

A comparison of the cancer risks depicted in Table 5 and in Table 6 shows that the major part of this risk is caused by amines which leach as part of an azo dye, and not by leaching of the "free" amine (contribution of "free" amine to the cancer risk in 5 products: <1% to 10%; for 1 product: 24%). This difference is mainly caused by the much higher amount of azo dye which leaches from the product than "free" amine.

Table 5. Calculated cancer risk from consumer exposure to azo dyes in garment ("free" aromatic amines included)

Product\ Material\ Route of exposure	Amine*	$E_{eff,total\ amine}$ (ng/day)	Cancer risk ($x10^{-6}$)
Underwear unknown\dermal	benzidine	7.5	25
Blouse silk\dermal	*o*-tolidine	119	397
Children's legging cotton-elastine dermal	benzidine	59	197
String of children's sweater cotton\oral	*o*-dianisidine	6.4	5[&]
Underwear silk\dermal	benzidine	285	950
Lining of a children's coat polyamide\dermal	*o*-dianisidine	1.0	3
	o-tolidine	0.36	1
	benzidine	1.3	4

* Detected amine
\# Total amine, i.e. the sum of the amounts of "free" amine and amine as part of an azo dye
& Total leachable amount, i.e. the sum of the amounts of "free" amine and amine as part of an azo dye which leaches from the product with a sweat simulant
[&] calculated after correction for less than life-time exposure (see Appendix 1, Table 1).

Table 6. Calculated cancer risk from consumer exposure to "free" aromatic amines in garment.

Product\ Material\ Route of exposure	Amine*	$E_{eff,"free" amine}$ (ng/day)	Cancer risk ($x10^{-6}$)
Underwear	benzidine	1.8	6
Blouse	*o*-tolidine	< 1.6	< 5
Children's legging	benzidine	1.8	6
String of children's sweater	*o*-dianisidine	< 0.07	< 0.05[ll]
Underwear	benzidine	8.0	27
Lining of a children's coat	*o*-dianisidine *o*-tolidine benzidine	< 0.1 < 0.1 < 0.1	< 0.3 < 0.3 < 0.3

* as in Table 1
\# Amount of "free" amine
& Amount of "free" amine which leaches with sweat simulant
ll calculated after correction for less than life-time exposure (see Appendix 1, Table 2).

4.4 Cancer risk: Footwear

In the Netherlands benzidine, *o*-dianisidine and 2,4-toluenediamine have been found in footwear (Van Haperen, personal communication). The cancer risks which correspond with the occurrence of these amines in footwear are presented in Table 7 and in Table 8. Table 7 shows calculated cancer risks for the total amount of amine. Table 8 shows calculated cancer risk for the "free" amine only.

When the total amount of amine is taken into account the exposure to 3 of the products leads to a cancer risk which is above the NRL. When only the "free" amine is taken into account Table 8 shows that, possibly, the exposure to 1 of the products leads to a cancer risk above the NRL.

Note that at equal levels of azo dyes in products substantially more of the dye leaches with sweat simulant from leather products than from textile.

In concordance with the findings in clothing a comparison between Tables 7 and 8 shows that the major part of the cancer risk is caused by amines which leach as part of an azo dye, and not by leaching of the "free" amine.

Table 7. Calculated cancer risk from consumer exposure to azo dyes in footwear ("free" aromatic amines included)

Product\ Material\ Route of exposure	Amine*	$E_{eff,total\ amine}$ (ng/day)	Cancer risk ($x10^{-6}$)
Children's slipper textile\dermal	benzidine	3.0	10
Children's slipper textile\dermal	2,4-toluene diamine	0.41	1
Shoe (upper side) leather\dermal	benzidine	13.2	44
Sportsshoe (upper side) leather\dermal	*o*-dianisidine	4.8	16

* as in Table 1 # as in Table 1 & as in Table 1

Table 8. Calculated cancer risk from consumer exposure to "free" aromatic amines in footwear

Product\ Material\ Route of exposure	Amine*	$E_{eff,"free"\ amine}$ (ng/day)	Cancer risk ($x10^{-6}$)
Children's slipper textile\dermal	benzidine	0.0049-0.049	0.02-0.2
Children's slipper textile\dermal	2,4-toluenediamine	< 0.076	< 0.2
Shoe (upper side) leather\dermal	benzidine	0.18-1.8	0.6-6
Sportsshoe (upper side) leather\dermal	*o*-dianisidine	0.03-0.3	0.1-1

* as in Table 2 # as in Table 2& as in Table 2

4.5 Actual urinary cancer rates in the Netherlands

In order to verify the outcome of the present risk assessment in terms of predicted cancer rates, a comparison is made with actual registered rates for cancer of the urinary bladder, i.e. the site in humans associated with exposure to aromatic amines. For this purpose it is assumed that past exposures of the population to aromatic amines have been at least the level presented here.

According to the data of the Fourth Report of the Netherlands Cancer Registry (1992) the actual incidences are 216 new cases of urinary bladder per million (person-years) males and 59 new cases per million (person-years) females of the total Dutch population, resulting in an average incidence of 140 new cases per million persons per year using an 1:1 sex ratio. European standardized rates (ESR) are very similar: respectively 23.6 and 4.7 per 100,000 person-years, thus 236 new cases per million males per year and 47 new cases per million females per year, also resulting in an average incidence of 140 new cases per million persons per year. During the years 1985 till 1995 no significant changes in (European) standardized rates for both males and females have been reported. Smoking, occupational exposure to carcinogens, and infection with *Schistosoma haematobium* usually are considered as main causative factors.

The highest risk calculated in this report for consumers dermally exposed to garment (silk underwear, coloured with a benzidine-based azo dye) amounts to 950 extra cases per million persons lifetime. Based on a lifespan of 75 years (Appendix 2.1: Dutch method) and the assumption that all cases are urinary bladder cancer, this would correspond to 12.7 extra cases of urinary bladder cancer per million (person-years).

5. Discussion

5.1 Evaluation of assumptions underlying the exposure assessment

The results of this study indicate that the wearing of garment and footwear which contain carcinogenic azo dyes and " free" amines may lead to an exposure well above the NRL, and sometimes even the MPRL. This conclusion is based on a comparison of the calculated uptake of amines from garment and footwear and the NRL, resp. the MPRL. In order to calculate this uptake several assumptions had to be made, some of which bear more uncertainty then others. A right interpretation of the cancer risks presented in Tables 5-8 needs a systematic evaluation of this uncertainty. This evaluation is presented below.

Assumption of life long exposure

The calculations of the cancer risks assume that life-long exposure occurs to the analysed products. To what extent is this assumption justified? A number of the analysed products is worne by adults (underwear, blouse, footwear). Only when consumers buy these products during their entire life (near) life-long exposure to these products may occur. Whether or not this actually occurs needs specialised information on the long-term characteristics of consumer behaviour, in this case the purchasing of textile and leather products. Such information is not (yet) available. Consequently, it cannot be evaluated whether the assumed life-long exposure to these products represents a "realistic case" (real exposure nearly life time) or a "worst case" (real exposure far less than life-time) approach.

For some of the analysed products the assumption of life-long exposure seems, beforehand, rather unrealistic. Examples of these products are children's clothes. In this case only the assumption that the material in children's clothes may be used in adult clothing too makes a life-long exposure to such material realistic.

In one product (children's sweater) the exposure to a carcinogenic amine (*o*-dianisidine) takes place when the product is sucked on. This exposure is restricted to childhood. In order to calculate the life time cancer risk which corresponds to the wearing of this product the exposure to this specific product had to be corrected for its "less than life time" character. This correction was performed by assuming that cancer risk is proportional to the cumulative dose during the wearing of the product. Assuming childhood to span 15 years and life time to last for 70 years the life time cancer risk corresponding with the exposure to this product was obtained by correcting the cancer risk as calculated during childhood with a factor equal to 15/70 (also see Table 1 of Appendix 1).

Extraction with sweat simulant as model for leaching with sweat

The risk assessment in this report is based on the amounts of amines ("free" as well as part of an azo dye) which leach from garment and footwear with a sweat simulant. This leaching is experimentally determined by exposing 1 gram of the product for 16 hrs., at 37 °C to 100 ml of a sweat simulant. During this extraction the samples were well stirred. Though these

conditions garantee an optimal extraction of amines and azo dyes from products they differ from the conditions which prevail on the skin when these compounds leach with sweat. For, on the skin the amount of sweat is much smaller than the amount of the material which is being leached, whereas under the mentioned experimental conditions just the reverse situation occurs. Furthermore, sweat evaporates on the skin. This process will decrease the efficiency of leaching, especially at relative long exposure times. Finally, the used extraction time of 16 hrs. will, even under extreme conditions such as athletic endurance, not occur.

In conclusion it is expected that the experimentally determined extraction with sweat simulant overestimates the leaching with sweat. This uncertainty can only be reduced by extracting amines and, in particular, azo dyes from garment and footwear under physiologically more realistic conditions.

Contact Frequency

The estimation of the contact frequency needs information on the frequency with which consumers buy products and the frequency with which consumers come into contact with these products.

Regarding the frequency with which consumers buy garment and footwear a frequency of 1 per year was assumed. As with other aspects of consumer behaviour the uncertainty which adheres to this assumption can only be reduced when additional information on this particular type of consumer behaviour becomes available.

In the case of garment it was assumed that the exposure to "free" amines is limited to new, non-washed, material. The reason for making this assumption lies in the observation that "free" amines almost totally leach from textile the first time it is washed (Speijers *et al.*, 1996). For the leaching of "free" amines from clothing the frequency of exposure thus equals the frequency with which they are purchased. This also implies that the exposure to "free" amines from textile only spans a small part of the period during which textile is actually worne.

In the case of azo dyes it was assumed that the exposure to these compounds and the amines therein is not limited to newly bought garment. In the calculations a (mean) decrease of 5% was used for the amount of azo dyes which leaches from garment every time it is washed. From this it follows that the leaching of azo dyes is negligible for clothes which are washed for 20 times or more. For garment the (mean) exposure to azo dyes, i.e. the amount which is expected to reach the skin, thus is 10 times the amount of dye which leaches from the new, unwashed, product. For the leaching of azo dyes from clothing the contact frequency thus is equivalent to 10 times the frequency with which they are purchased.

In comparison with garment the exposure to azo-dye from textile footwear may be relative long. The reason for this is that, in contrast to garment (relative high leaching of dye during washing, relative low leaching with sweat during wearing), the amount of azo dyes in textile footwear only declines from leaching with sweat, a relative inefficient process. However, in combination with a relative high contact frequency (daily exposure during a product cycle of 1 year) it was calculated that 25 to almost 100 % of the dye which is present in textile footwear may, eventually, leach with sweat from the product. A similar conclusion

was drawn for leather footwear. The high leaching of azo dyes with sweat from leather footwear indicates that already after several times of wearing all azo dyes which are present in leather footwear may have leached from the product.

Fraction absorbed

For amines a complete dermal and gastro-intestinal absorption was assumed. For azo dyes this fraction was assumed to be 10% for the dermal route and 100% for the gastro-intestinal route. These assumptions are based on experimental observations with one amine (benzidine) in the rat and with three azo dyes in *in vitro* experiments with human skin. Being experimentally determined with rather high accuracy these fractions bear relative low uncertainty.

Skin contact factor

In attributing the skin contact factor a discrimination was made between direct and indirect skin contact. An example of a product which only directly comes into contact with the skin is underwear. The fact that this product is directly worne on the skin justifies a skin contact factor of 1. On the other hand, footwear only indirectly comes into contact with the skin. For, usually, footwear is worne in combination with socks or stockings. This shields the skin from the products. This effect, of course, greatly depends on the type of socks/stockings worne. Whereas thin stockings will not provide much shielding (skin contact factor $\approx$ 1) the reverse may be true for socks made of wool (skin contact factor $\approx$ 0). As a gross, provisional, estimate a value of 0.1 was used for the skin contact factor of footwear. To assess this factor more accurately experimental research is needed on the extent to which textile and synthetic materials shield the skin from azo dyes and amines with leach from garment and footwear with sweat.

Probability of azo dyes and aromatic amines appearing in products

The chance of azo dyes and aromatic amines appearing in clothing (0.08, see Appendix 1) and footwear (0.10, see Appendix 1) is based on the frequency with which these compounds were found in the Netherlands during sampling of lots of these products which are available on the Dutch market. The value for the chance in clothing is lower than reported for the German (0.14, as mentioned in RPA, 1997) and the Danish market (0.24, as mentioned in RPA, 1997; 0.56, Wallin *et al.*, 1998).

During sampling a positive selection for products which contain azo dyes and amines inevitable has taken place. So, the true chance of azo dyes and amines appearing in products which are available on the market is lower than than used in the calculations in this report.

5.2 Uncertainties in the dose-response assessment

The method which was used to derive a limit value for benzidine and which was also applied for the other aromatic amines has the following uncertainties.

First of all, the cohort considered by U.S.EPA is quite small, i.e. only a total of 25 benzidine workers have been monitored. Secondly, there are some questions about the accuracy of the applied monitoring method for benzidine, i.e. by making use of the Chloramine-T reagent (Zavon *et al*, 1973). It is unclear how specific this assay is towards benzidine, and whether other chemical exposures may have interfered in this; this even may concern chemicals that might be bladder carcinogens themselves as well. But it is also unclear whether acetylated benzidine metabolites, representing over 95% of total human urinary benzidine excretion (Rothman *et al.*, 1996), are detected in this analysis. A third source of uncertainty is the concept used by U.S.EPA in their hazard characterization approach: i.e. urinary benzidine levels are taken as a valid surrogate for the effects observed, irrespective of the route of exposure. Clearly, benzidine levels in urine may well represent the exposure to benzidine of the urinary bladder epithelium, the target tissue for carcinogenesis. However, there is clear evidence that benzidine-metabolites are the ultimate carcinogenic species, and that these are thought to be generated outside the bladder epithelium, i.e. mainly in the liver (Chou *et al.*, 1995). Thus, it can be anticipated that different exposure routes may result in quite different amounts of critical metabolites in the urinary bladder lumen, implying in parallel quite different cancer responses as well. Clearly, the last two points of uncertainty have substantial influence on the limit value finally derived at.

In this context also the remark made by IARC on this specific cohort should be noticed. In their Monograph series (IARC, 1982) IARC states: "Of the epidemiological studies available to the Working Group, only that of Zavon *et al.* (1973) provides sufficient information on levels of exposure to benzidine to serve as a basis for quantitative risk assessment. This study allows very crude estimates of the carcinogenic potency of benzidine to be made."

From the above it is clear that it is highly desirable to resolve, or at least diminish, the addressed uncertainties. For this, essentially two ways are open, that both should be explored: first, to further evaluate the validity of the applied exposure monitoring method in the study of Zavon *et al.* (1973) and, secondly, to review more recently published data, especially in the field of epidemiology. These issues will be addressed in a discussion which has recently been started with the Dutch Expert Committee on Occupational Standards of the Health Council of the Netherlands.

Finally, it has to be mentioned that a risk assessment based on the extrapolation of the carcinogenicity of benzidine from animals to man would have a great impact on the conclusions as drawn in this report. For, if the observed TD_{50} for benzidine in the rat bioassay is taken as a point of departure for such an extrapolation (which concerns an inhalation study; Gold and Zeiger, 1997), we would arrive at an acceptable 10^{-6} cancer risk level of 238 ng

benzidine per person per day, i.e. an about 800-fold lower risk as compared to that derived at by U.S. EPA from human data. In this case only one calculation, i.e. the one concerning underwear with the highest benzidine level, would lead to a cancer risk which is just above the NRL.

5.3 Comparison of methods for estimating the carcinogenic amines

In Appendix 3 a comparison is made between the calculation of the uptake of benzidine and *o*-tolidine from two pieces of clothing (underwear; blouse) as made with the method depicted in Fig. 2 and the LGC-method (Fig. 1). The LGC-method calculated for these products uptakes of 710 ng benzidine and 1090 ng *o*-tolidine per person per day. With the method shown in Fig. 2 the calculated amounts were 285 ng benzidine and 119 ng *o*-tolidine per person per day (see Table 1). So, the uptake of these amines as calculated with the current method was 2.5 and 9-fold lower than calculated with the LGC method. The observed difference is caused by differences in the assumptions on the rate at which azo dyes migrate (LGC-method) from products onto the skin. For example, the calculations with the LGC-method which are shown in the Appendix were performed while assuming a rate of migration of azo dyes of 0.01%/hr. However, depending on the azo dye used (direct, basic, acid, reactive or disperse) migration rates which vary from 0.001 to 0.01%/hr may be used instead (LGC, 1998). Or, in other words, had the lowest value of this range been used for the calculations with the LGC-method the uptake of amines as calculated with this method would have been in the same order as with the method shown in Fig. 2.

5.4 Final conclusions

In this report a cancer risk assessment is presented for garment and footwear which contain azo dyes and aromatic amines. This assessment is based on the experimentally determined amount of amines which leaches with sweat simulant from products. With this method cancer risks were calculated for 7 pieces of garment and 4 pieces of footwear (2 textile and 2 leather pieces). In all cases cancer risks well above the NRL were calculated. In some cases the calculated cancer risk even exceeded the MPRL. Cancer risks were mainly determined by leached azo dyes, with leached "free" amines only marginally contributing.

In the proposed method the experimental set up for the determination of the leaching of azo dyes and "free" amine with a sweat simulant from garment and footwear overestimates the leaching of these compounds during sweating. This has led to an overestimation of the cancer risk which results from the wearing of garment and footwear which contains such compounds. Another factor which contributes to this overestimation is the assumed probability with which azo dyes and aromatic amines occur in garment and footwear (the available information inevitable leads to an overestimation of this chance). On the other hand, the way the indirect exposure to azo dyes and amines was quantified may, especially in the case of footwear, have led to underestimated cancer risks.

When the above mentioned considerations are taken into account it has to be concluded that the cancer risk assessment in this report overestimates cancer risks by one, or even more than one order of magnitude.

5.5 Towards a more "realistic case": Recommended research

In order to improve the risk assessment presented in this report the following research is recommended.

Exposure assessment

- experimental research on the leaching of amines and, in particular, azo dyes under physiologically relevant conditions, i.e. under conditions which mimic the leaching of these compounds from the wearing of these articles. Furthermore in this research the effect of textile and synthetics on the shielding of the skin from amines and azo dyes which leach from products with sweat should be addressed.

- research on the purchasing behaviour on consumers and the probability that garment and footwear contain carcinogenic azo dyes and aromatic amines

Effect assessment

- a reanalysis of US. EPA's cancer risk assesment of benzidine

- comparative analysis of relevant DNA adduct formation in most suitable rodent and human; in the end together with PBPK modeling

- verification of cancer risks via epidemiological studies focussing on exposure assessment in parallel with investigations on tumour precursor lesions.

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Appendix 1 Exposure Assessment

This appendix describes the calculation of the exposure of consumers to azo dyes and aromatic amines from garment and footwear.

In general the exposure to carcinogenic aromatic amines from garment and footwear has two components. First amines may leach from garment and footwear as part of azo dyes. After leaching from the product onto the skin the amine may be split off from the azo dye by metabolic conversion. This conversion may be perfomed by bacteria present on the skin or in the gastro-intestinal tract or by mammalian enzymes in the skin. Furthermore, garment and footwear may contain amines in their so-called "free", i.e. unbound, form. These "free" amines also may leach with sweat onto the skin. Factors which are taken into account in the calculation are:

- the chance that garment and footwear which comes into contct with the skin contain "free" amines and amine containing azo dyes
- the frequency with which consumers come into contact with garment and footwear which contain azo dyes and amines(contact frequency)
- The fraction of garment and footwear which actually comes into contact with the skin (skin contact factor)
- The metabolic conversion of leached azo dyes into their constituting amines in/on the skin and in the gastro-intestinal tract.
- The absorption of amines and azo-dyes (fraction absorbed).

The experimentally determined amount of leached amine was used as the starting points for the calculation of $E_{eff,total\ amine}$. This calculation is represented by the following equation:

$$E_{eff,total\ amine} = P \times F_{cont.} \times A_{amine} \times W \times F_{skin} \times F_{abs}$$

$E_{eff,total\ amine}$	Expected daily uptake of amine (sum of "free" amine and amine which is part of an azo dye) (g/day)
P	Probability that a product contains a carcinogenic azo dye, cq. carcinogenic amine (no dimension).
$F_{cont.}$	Contact frequency (day^{-1})
A_{amine}	The amount of amine which leaches with sweat simulant from one gram of a product (sum of the leached amount of "free" amine and the amount of amine which leaches as part of an azo dye, g/g)
W	Weight of dye/amine containing material (g)
F_{skin}	Skin contact factor (no dimension)
F_{abs}	Fraction absorbed (no dimension).

Garment

The chance that garment contains azo dyes and aromatic amines

Not all garment which is available on the Duch market contains carcinogenic azo dyes and aromatic amines. In the Netherlands the consumer's organisation *Consumentenbond* found these compounds in 7 out of 100 investigated specimen of garment (n = 200, Luijk, 1996, as cited in CREM, 1997). Similarly, percentages of 11 (n ≈ 792, TNO, 1997), 7 (number of investigated specimen unknown, TÜV-Rheinland, 1997) en 6 have been reported (n = 116, *Keuringsdienst van Waren Alkmaar*, 1997). On the mean these investigations indicated a chance of 8% for the appearance of carcinogenic azo dyes and aromatic amines in garment.

Note

In sampling lots of garment which are available on the Dutch market, inevitably, positive selection for products which contain carcinogenic azo dyes and amines has taken place. So, the mentioned chance of 8 % is expected to overestimate the fraction of azo-dye containing garment.

Contact frequency

The frequency of contact is the number of times that consumers come into contact with garment which contains azo dyes and aromatic amines. For example, when garment is bought once a year (frequency of purchase: 1 per year; duration of use: 1 year), the purchased garment is worne daily and the leaching of azo dyes and amines with sweat is more or less constant over time the contact frequency is 365 per year.

To assess the contact frequency one needs, among others, to know the frequency with which garment is purchased by consumers. Unfortunately such information is not (yet) available. For the time being we therefore sufficed with assuming that, on the mean, consumers purchase garment at a frequency of 1 (example: coat) to 3 (example: underwear) specimen per year.

The reasoning held sofar assumes that the exposure to azo dyes and amines occurs every time that contact between the skin and garment takes place. In the case of garment this assumption certainly does not hold. For, during its period of wearing garment is regularly washed. As mentioned in Speijers *et al.* (1996) more than 80% of aromatic amines are removed from garment the first time it is washed. In calculating the exposure to "free" amines it was therefore assumed that the exposure to these compounds from garment is limited to newly bought, unwashed garment. Consequently, given a frequency of 1 per year for the purchase of garment, the (effective) contact frequency is set at 1 per year[9].

Contrary to "free" amines azo dyes may leach from garment which has several times been washed. Experiments performed by ETAD (ETAD, 1997) showed that, after more than 20 times of washing, the leaching of azo dyes from garment is negligible and declines with

[9] Note that when newly bought garment is washed before it is worne no exposure to "free" amines takes place.

almost 5% every time garment is washed. This means that, on the mean, the exposure of consumers to azo dyes from garment is equal to 10.5, rounded 10, times the amount which leaches from the newly bought, unwashed, product[10]. Given a frequency with which garment is purchased of 1 per year, a duration of use of 1 year and one time of wearing between two times of washing the (effective) contact frequency to azo dyes from garment is equivalent to 10 times the exposure to the newly bought product.

Leaching of amines and azo dyes with sweat

The leaching of amines and azo dyes was experimentally determined by extracting 1 gram of newly bought unwashed, garment with a sweat simulant (extraction conditions: 16 hrs., 37°C in 100 ml NaCl/phosphatebuffer, pH 6.8 while shaking). The total amount of amines and azo dyes which leaches from the product was calculated by multiplication of the experimentally determined amount per unit weight with the weight of the specific product.

Skin contact factor

In general the part of garment which comes into contact with the skin is variable. For example, underwear will completely come into contact with the skin (skin contac factor: 1). However, in the case of a coat's lining this will not be the case. For, usually only the ends of a coat's lining and the part of the coat which covers the neck directly comes into contact with the skin. So, for such a product only a small fraction will directly come into contact with the skin. This fraction is (arbitrarily) set at a maximum of 0.1. Consequently, the remaining fraction of the product comes indirectly into contact with the skin (mechanism: production of sweat, diffusion of sweat through garment, leaching of azo dyes and amines from the product, back diffusion of leached amines and dyes to the skin). It is assumed that the exposure to azo dyes and amines which result from indirect skin contact of garment is one order of magnitude lower than that of direct contact. For the lining of a coat this results in a (weighted, maximum) skin contact factor of $0.1 \times 1 + 0.9 \times 0.1 = 0.19$.

A number of products do not belong of either of the two categories mentioned before , i.e. almost exclusive direct or indirect skin contact. A typical example of such a product is a blouse. Mostly this product is worne in combination with underwear, the latter shielding the skin from the blouse. Assuming the fraction of the blouse which directly comes into contact with the skin between 0.1 (almost complete shielding of underwear) and 1 (no shielding of underwear) a mean fraction of 0.5 may be calculated for the fraction of the blouse which directly comes into contact with the skin. For this product a (weighted, maximum) skin contact factor of $0.5 \times 1 + 0.5 \times 0.1 = 0.55$ was calculated.

[10] This conclusion implies that azo dyes mainly disappear from garment as a result of washing, and not so much by leaching with sweat or saliva. The relative inefficient leaching of azo dyes with a sweat simulant (see Table 1 of the main text) supports this conclusion.

Fraction absorbed

For amines the fraction absorbed is defined as the fraction which, when present on the skin or in the gastro-intestinal tract, is taken up in the body. For azo dyes the fraction absorbed is defined as the fraction of the azo dye which, when present on the skin or in the gastro-intestinal tract, is taken up in the body in the form of its constituting amine.

In the rat orally administered benzidine is completely absorbed (Lynn *et al.*, 1984, vehicle: aquaous solution). In this species 50 % of dermally administered benzidine is absorbed within 24 hrs. after administration (Shah en Guthrie, 1983, vehicle: acetone). Given these absorption percentages the fraction absorbed of orally and dermally administered benzidine (and other aromatic amines) was set at 1.

6 days after the dermal administration of the radioactivily labeled azo dye DB38 to rabbits 8% of the administered label was excreted with the urine and the faeces (vehicle: aquaous solution). In the rat this percentage was found to be much lower (DB38: 0.2%; DB19: 0.05% en 0.04%, Aldrich *et al.*, 1986). Though these experiments did not reveal the nature of the excreted radiolabel, i.e. no discrimination was made between the parent azo dye , the aromatic amine and/or their metabolites, their results suggest the dermal uptake of the azo dye. Experiments performed by Collier *et al.* (1993) indeed showed a substantial uptake of azo dyes in human skin. When human skin was exposed *in vitro* to the azo dyes ANSC, Sudan I en Solvent Yellow 7 5, 30 and 35% of the adminisistered amounts were taken up within 24 hrs. (vehicle: acetone/methanol). In this period 30 and 27% of the absorbed amounts of Sudan I en Solvent Yellow 7 were converted into their constituting amine and amine metabolites (for rodent skin these percentages ranged from 5 to more than 60% for the uptake and from 50 to 60% for the conversion of the azo dye taken up).

The uptake of azo dyes across the wall of the gastro-intestinal tract is unknown. However, the gastro-intestinal tract possesses a rich flora of bacteria which can split amines from azo dyes (zie b.v. Chung, 1983). The substantial residence time of azo dyes in the gastro-intestinal tract and the high bacterial capacity therin to reduce azo dyes to their constituing amines justifies an absorption faction of 1 for amines which enter the gastro-intestinal tract via the oral route as part of an azo dye.

In summary the results mentioned above indicate that the absorbed fraction of orally and dermally administered aromatic amines is 1. For azo dyes (maximally) 30% of the amount whch leaches with sweat may be taken up by the human skin. Of this amount almost 30% is metabolised to amines. Or, in other words, about 9% of the amount of azo dyes which leach from a product onto the skin eventually will enter the body in the form of an aromatic amine. Hence, the (overall) fraction absorbed for the dermal route of the exposure to azo dyes was set at 0.09, rounded 0.1. For the oral route of exposure this fraction was set at 1.

As examples two calculations of the uptake of benzidine from underwear are presented. The first calculation applies to total benzidine, i.e. the sum of "free" benzidine and benzidine which is part of an azo dye. The second calculation presents the uptake of "free" benzidine.

Calculations for all products together are presented in Tables 1 and 2.

Calculation 1 (see also Table 1)

Probability of azo dye appearing	0.08
Leachable amount of benzidine as part of an azo dye (new, unwashed product)[11]	0.63 µg/g
Product weight	18 grams
Contact frequency (frequency of purchase: 3/year)	30/year
Skin contact factor	1
Calculated exposure to benzidine: 0.08 x 0.63 x 18 x 30 x 1 = 27.2 µg/year	
Fraction absorbed	0.1
Calculated uptake of benzidine 27.2 x 0.1 x 1000 = 2712 ng/year	

This is equivalent to a daily uptake of 7.5 ng benzidine[12].

Calculation 2 (see also Table 2)

Probability of benzidine appearing	0.08
Leachable amount of "free" amine (new, unwashed product)	0.09 µg/g
Product weight	30 grams
Contact frequency (frequency of purchase: 3/year)	3/year
Skin contact factor	1
Calculated exposure to benzidine: 0.08 x 0.09 x 30 x 3 x 1 = 0.65 µg/year	
Fraction absorbed	1
Calculated uptake of benzidine: 0.65 x 1000 = 650 ng/year	

This is equivalent to 1.8 ng benzidine per day[13].

[11] Amount in product which had once been washed: 0.6 µg/g. Assuming a 5% decrease of the azo dye every time the product is washed gives the amount in the new, unwashed product as 0.6/0.95 = 0.63 µg benzidine/g.
[12] to be compared with 1.8-890 ng/dag,(Speijers *et al.*, 1996).
[13] to be compared with 0.74-37 ng/dag (Speijers *et al.*, 1996).

Table 1. Expected uptake of amines after the exposure to clothing containing "free" aromatic amines and amine containing azo dyes

Product/ Material/ Route of exposure	Amine*	Amount# (µg/g)	Leachable amount& (µg/g)	Contact frequency ($year^{-1}$)‡	Amount leached (µg/g)	Weight (g)	Fraction absorbed	Skin contact factor	Chance of azo dye occurring	Calculated amine uptake (µg/jaar)	Calculated amine uptake (ng/dag)
Underwear/ unk./dermal	benzidine	1368§	0.63	30	18.9	18	0.1	1	0.08	2.7	7.5
Blouse/ silk/dermal	o-tolidine	2796@	3.8 (3.6;3.9)	20	76	130	0.1	0.55	0.08	43.5	119
Children's legging/ cotton-elastine/ dermal	benzidine	1112@	1.8 (2.3;1.3)	20	36	75	0.1	1	0.08	21.6	59
String of a children's/ sweater/ cotton/oral¶	o-dianisidine	337@	0.49	20	9.8	3	1	1	0.08	2.4	6.4
Underwear/ silk/dermal	Benzidine	3138@	5.7 (5.2;6.1)	30	171	76	0.1	1	0.08	104	285
Lining of a children's coat/ dermal/polyamide	o-dianisidine	110@	0.50 (0.44;0.55)	10	5.0	50	0.1	0.19	0.08	0.38	1.0
	o-tolidine	35@	0.18 (0.13;0.22)	10	1.8	50	0.1	0.19	0.08	0.14	0.36
	benzidine	17@	0.63 (0.56;0.70)	10	6.3	50	0.1	0.19	0.08	0.48	1.3

Legend to Table 1

* Detected amine
# Amount of amine detected in product, i.e. the sum of "free'amine and amine which is part of an azo dye
& Amount of amine, i.e. the sum of "free" amine and amine which is part of an azo dye which leaches from a new, unwashed product (phosphate buffer pH = 6.8; 16 hrs., 37°C)
$ extraction method: 1% ammonia, 4 hrs., 60°C
@ extraction method: citratebuffer pH = 6, 30 min, 70°C
⅃ frequency with which the product is purchased: 1-3/year 1: children's coat, 2: blouse, children's legging, children's sweater, 3: underwear; maximal number of exposures during the life cycle of the product: 10
⅂ calculated after correction for less than life-time exposure. Oral exposure to o-dianisidine occurs by sucking on the product, an exposure which is confined to childhood (set at maximally 15 years; life time: 70 years). The calculated cancer risk therefore is $15/70 \times 6.4 /0.3 = 4.6 \times 10^{-6}$
¶ exposure by sucking, leaching with sweat and with saliva assumed to be equal.

For each of the products studied the calculated amount of the amine which leaches onto the skin during the period of wearing only is minor in comparison with the the amount of amine which is present in the product. For example, in underwear 1368 µg benzidine per gram of product was found. The amount of benzidine which may leach from this product was calculated to be 10 x 0.63µg/g/year, or 10 x 0.63/1368 x 100% = 0.46% of the amount present in the product. For percentages corresponding with the other products were 1.4; 1.6 ; 1.5; 1.8; 4.5; 5 and 37. So, with one exception, these percentages were low because of the relative low leaching with sweat and the low exposure frequency.

Table 2. Expected uptake of "free" amines from garment

Product	Amine*	Amount (µg/g)#	Leachable amount& (µg/g)	Contact frequency ($year^{-1}$)‖	Amount leached (µg/g)	Weight (g)	Fraction absorbed	Skin contact factor	Chance of amine occurring	Calculated amine uptake (µg/jaar)	Calculated amine uptake (ng/dag)
Underwear	benzidine	unknown	0.09	3	0.27	30	1	1	0.08	0.65	1.8
Blouse	o-tolidine	unknown	< 0.05	2	< 0.1	130	1	0.55	0.08	< 0.57	< 1.6
Children's legging	benzidine	unknown	0.055 (0.05;0.06)	2	0.11	75	1	1	0.08	1.8	0.66
String of a children's sweater	o-dianisidine	unknown	< 0.05	2	< 0.10	3	1	1	0.08	< 0.024	< 0.07
Underwear	benzidine	unknown	0.16 (0.08;0.14)	3	0.48	76	1	1	0.08	2.9	8.0
Lining of a children's coat	o-dianisidine	unknown	< 0.05	1	< 0.05	50	1	0.19	0.08	< 0.038	< 0.10
	o-tolidine	unknown	< 0.05	1	< 0.05	50	1	0.19	0.08	idem	idem
	benzidine	unknown	< 0.05	1	< 0.05	50	1	0.19	0.08	idem	idem

* as in Table 1

Amount of "free" amine

& Amount of "free" amine which leaches with sweat simulant

‖ frequency with which the product is purchased: see Table 1; maximal number of exposures during the life cycle of the product: 1.

Footwear

As with garment it is assumed that the exposure to azo dyes and aromatic amines from footwear takes place by leaching with sweat.

The CREM report mentions, on the basis of data reported by TÜV Rheinland and TNO, percentages of 15 (number of analysed specimen unknown) and 5 (1 out of 20 analysed specimen) for the frequency with which carcinogenic azo dyes and amines appear in footwear. So, a mean of 10% was taken for this frequency.

In contrast to garment, in which the decrease of the amount of azo dyes is mainly determined by the number of times with which garment is washed, this decrease in footwear is solely determined by leaching with sweat. Experimentally the leaching of azo dyes from leather footwear appeared very efficient, a single extraction with a sweat simulant removing 15 and 39% (see Table 3 of the main text). This means that, in practice, the exposure to azo dyes from newly bought footwear is limited to the first times it is worne. Or, in other words, it may be expected that all azo dyes present in leather footwear have leached with sweat after several times of wearing.

In the case of textile footwear azo dyes showed much lower extractability with sweat, with only 0.1 to 1% of the amount of dye in the product leaching after a single extraction with a sweat simulant (see Table 3 of the main text). When it is assumed that footwear is yearly purchased and worne daily it is expected that after one year 25 and 97 % of the amount initially present in these products has leached with sweat.

In the case of textile garment the good water solubility of amines garantees an efficient leaching when textile is washed. As a result the exposure to amines only takes place during the wearing of new, unwashed garment. In the case of footwear the removal of amines from the product by washing does not occur. However, given the good water solubility of these compounds it is expected that the leaching of "free" amines from footwear will be limited to, say, 1 to 10 times it is worne.

As with garment a skin contact factor of 1 indicates that footwear has direct contact with the skin. In practice this means that footwear is always worne on the naked skin. This assumption of course is not very realistic. For, footwear is usually worne in combination with socks or stockings, a situation corresponding with indirect skin contact (mechanism: sweating, diffusion through socks of stockings, leaching of footwear with sweat, back-diffusion of lached amines and azo dyes to the skin, skin contact factor $0 \times 1 + 1 \times 0.1 = 0.1$).

For one of the specimen of footwear the above mentioned reasoning does not hold. In this product, i.e. a sportsshoe, the skin is, next to socks or stockings, shielded from the part of the shoe which contains the azo dye and its constituting amine by several layers of textile and (dye free) leather. This (extra) shielding of the skin is accounted for by assuming it to reduce the efficiency of the leaching with sweat by one (extra) order of magnitude. Hence for this particular product a skin contact factor of $0 \times 1 + 1 \times 0.01 = 0.01$ was calculated.

The fraction absorbed of leached amines and dyes from garment and from footwear were assumed to be the same.

Calculations of the uptake of amines from footwear are presented in Tables 3 and 4.

Table 3. Expected uptake of amines after the exposure to azo dyes in footwear ("free" aromatic amines included)

Product/ Material/ Route of exposure	Amine*	Amount (µg/g)#	Leachable amount (µg/g)&	Contact frequency ($year^{-1}$)	Amount leached (µg/g)	Weight (g)	Fraction absorbed	Skin contact factor	Chance of azo dye occurring	Calculated amine uptake (µg/year)	Calculated amine uptake (ng/day)
Children's slipper/ Textile/dermal	benzidine	381	3.8	365	370	3	0.1	0.1	0.1	1.1	3.0
Children's slipper/ Textile/dermal	2,4-toluenediamine	210	0.17	365	53	2.8	0.1	0.1	0.1	0.15	0.41
Schoe (upper side)/ Leather/dermal	benzidine	161	24	365	161	30	0.1	0.1	0.1	4.8	13.2
Sportschoe (upper side)/ leather/dermal	o-dianisidine	589	231	365	589	30	0.1	0.01	0.1	1.8	4.8

* as in Table 1 # as in Table 1 & as in Table 1 ⅄frequency with which the product is purchased: 1/year, maximal number of exposures during the life cycle of the product: 365. For three of the four products studied the calculated amount of the amine which leaches onto the skin during the period of wearing is equal to the amount of amine which is present in the product. For one product this percentage amounted 30. These percentages are high because of the high leaching with sweat.

Table 4. Expected uptake of "free" amines from footwear

Product	Amine*	Amount (µg/g)#	Leachable amount (µg/g)&	Contact frequency ($year^{-1}$)‡	Amount Leached (µg/g)	Weight (g)	Fraction absorbed	Skin contact factor	Chance of amine ocurring	Calculated amine uptake (µg/year)	Calculated amine uptake (ng/day)
Children's slipper	benzidine	onb	0.06	1-10	0.06-0.6	3	1	0.1	0.1	0.0018-0.018	0.0049-0.049
Children's slipper	2,4-toluenediamine	onb.	< 0.1	1-10	< 1	2.8	1	0.1	0.1	< 0.028	< 0.076
Schoe (upper side)	benzidine	onb.	0.22	1-10	0.22-2.2	30	1	0.1	0.1	0.066-0.66	0.18-1.8
Sportschoe (upper side)	o-dianisidine	onb.	0.35	1-10	0.35-3.5	30	1	0.01	0.1	0.01-0.1	0.03-0.3

* as in Table 2 # as in Table 2& as in Table 2 $ carc. potency of 2,4-toluenediamine equal to benzidine ‡ frequency with which the product is purchased: 1/year, maximal number of exposures during the life cycle of the product: 365.

Appendix 2 Effect Assessment

2.1 - Derivation of the $1x10^{-6}$ (lifetime) risk level for benzidine (United States method and Dutch Method)

In this appendix calculations of the risk level of $1x10^{-6}$ lifetime, i.e. one extra case of cancer per milion persons exposed lifetime, underlying the data in section 3.2.1 in Chapter 3, are presented. This risk level is referred to in literature as the "virtual safe dose (VSD) or the "acceptable risk level". In Dutch policy this risk level is considered to be the "negligible risk level" (NRL). A risk level that is 100 times higher ($1x10^{-4}$ lifetime, i.e. one extra case of cancer per 10.000 persons exposed lifetime) is considered to be the "maximum permissible risk level"(MPRL).

'Derivation of VSD' by U.S. EPA
(U.S. EPA, 1997)

Available data:
observed bladder tumour incidence: 52% (13 out of 25)
mean duration of employment (exposure): 11.46 years
240 working days/year
mean age at cohort: 56.5 year
urine level of benzidine in workers (at end of workshift): 0.04 mg/l
urine production per worker per day: 1.2 l
recovery factor of exposure in urine: 1.45% (from monkey study, Rinde and Troll, 1975)average lifespan: 71.3 years

Calculation:
lifetime exposure: 0.0063 mg/kg/day
inhalation slope factor calculated: 2.3 10^2 per (mg/kg/day) (via one-hit model with time factor, extra risk)
linear extrapolation from this factor to an additional one per million risk: 0.3 ng per person per day (person of 70 kg)

'Derivation of VSD' via Dutch method
(HCN, 1994)

Available data:
similar as above; proportional corrections made for 5 to 7 days a week, and for observed exposure-period to lifetime exposure, which is 75 years in the NL.

Calculation:

daily excretion of benzidine in urine: 0.048 mg
daily exposure, 5 days a week: 3.31 mg per person (from 1.45% being excreted)
exposure correction for 5 to 7 days a week during employment: 2.18 mg per person (i.e. 7 days a week)
exposure correction for 11.46 to 56.5 years, daily: 0.44 mg per person
exposure correction for 56.5 to 75 years, daily: 0.33 mg per person
observation correction for 56.5 to 75 years, daily: 0.25 mg per person
life-time10^{-6} risk related exposure: $10^{-6}/0.52$ x 0.25 = 0.48 ng per person in NL (note ; 0.52 was observed incidence)
(corresponding figure for US lifespan of 71.3 years with this methodology is 0.53 ng per person, daily)

2.2 TD_{50}

The TD_{50} is defined as the dose rate in mg per kg body weight per day required to halve the probability of an experimental animal of remaining tumour free by the end of the standard life-span for the species (Peto *et al.*, 1984, Sawyer *et al.*, 1984). The group of Peto and Sawyer have worked out in detail two methods of statistical analysis of bioassay results: one using life table data and the other summary incidence data. From either type of analysis the carcinogenic potency is estimated as TD_{50}. The analysis using life table data is far more complex than the summary incidence method, which does not take into account correction for mortality. Comparison of the two methods has shown that there is substantial agreement between the summary incidence and the life table methods of analysis in terms of potency estimation (Gold *et al.*, 1986).

In order to provide the most accurate estimates of the TD_{50} and to provide a large resource with comparable data to facilitate the analysis of the results of animal cancer tests, Gold *et al.* calculate the TD_{50} in a standardized way using a number of strictly defined starting points regarding 1) selection of tissues and tumour types for calculation of carcinogenic potency, 2) the average daily dose level and 3) the extrapolation of TD_{50} to standard life-span of the species, etc. (Gold *et al.*, 1984).

Criteria for selection of studies

The following set of standard criteria is used for selection of studies: a) animals on test were mammals, b) administration was begun early in life, c) route of administration was diet, water, gavage, inhalation, intravenous or intraperitoneal injection, d) test agent was administered alone, e) exposure was chronic, with not more than 7 days between administrations, f) duration of exposure was at least one-fourth the standard lifespan for that species, h) research included a control group, i) research design included at least 5 animals per group, j) pathology data were reported for the number of animals with tumours rather than the total number of tumours, k) results reported were original data.

Estimation of daily dose rate in mg/kg bw/day and correction of the TD_{50} for the possible difference in experimental period and standard lifespan

TD_{50}s are expressed in mg/kg bw/day irrespective of the way of administering the test substance. So, also TD_{50}s derived from inhalation experiments are given in mg/kg bw/day. By assuming 100% absorption, and adopting a set of standard values for each sex/species group which includes factors for daily food, water and air intake and average weight, dose is converted to mg/kg bw/day. Dose rate = [dose x intake x number of doses per week] : [animal weight x 7 days]. In many experiments the administration of the test compound is stopped before the terminal sacrifice or before the death of the last animal. By convention the total dose administered is spread over the entire experimental period. In an experiment which is terminated before the standard lifespan, the number of tumours found will be lower resulting in an underestimation of the TD_{50}. For this reason the true TD_{50} is estimated as f^2d, where f = [duration of experiment] : [standard lifespan], and d = dose rate.

In summary, Gold *et al.* discern three different periods, i.e., exposure time (Xpo), experimental time (Xpe) and lifespan of the animal (L), which may lead to one of the following situations:

1) Xpo = Xpe = L, no correction indicated;
2) Xpo < Xpe = L, correction d x Xpo/Xpe;
3) Xpo = Xpe < L, correction d x $(Xpe/L)^2$;
4) Xpo < Xpe < L, correction d x Xpo/L x Xpe/L

Fields of application of the TD_{50}

TD_{50} values for more than 1000 different chemicals have been published by Gold *et al.* in their ongoing Carcinogenic Potency Database (CPDB) (Gold *et al.*, 1984, 1990, 1993, 1995). TD_{50}s are reported for the different target organs/tissues separately, for combinations of selected target tissues/organs, and for the number of animals with treatment-related tumours.

The TD_{50} approach has among others been used for ranking and classification of carcinogens according to potency, for setting priorities, also in combination with human exposure estimates: HERP, Human Exposure dose/Rodent Potency dose (Ames *et al.*, 1987). Ames *et al.* (1987) introduced the HERP as a measure of possible carcinogenic hazard to man. The HERP is based on estimated lifelong daily exposure of man and the TD_{50} value estimated from rodent studies, and is calculated by expressing human exposure (daily lifetime dose in mg/kg bw) as a percentage of the rodent TD_{50} (in mg/kg bw). The HERP is not intended to be used as a direct estimate of human hazard but merely for setting priorities (Ames *et al.*, 1987).

2.3 Peak vs. repeated exposure: Dose-Rate Correction Factor (DRCF)

Health-based recommended life-time exposure limits for genotoxic compounds are obtained from dose-response data obtained in chronic animal experiments or, occasionally, from epidemiological data. Thus, the exposure limits actually address lifetime daily low-dose exposures. However, from the exposure assessment chapter it is clear that the exposure to carcinogenic amines from textiles and footwear in most cases merely has 'peak exposure' characteristics. In this context 'peak exposure' is defined as a single, relative short (lasting less than 24 hours) exposure to a relative high dose of the substance. A 1994 report of the Health Council of the Netherlands (NHC, 1994) has tried to address the question what cancer risks are associated with peak exposure to a genotoxic carcinogen (i.e relative to the cancer risk of the same dose of this carcinogen when distributed over an entire lifespan). For this a so-called Dose-Rate Correction Factor (DRCF) was defined as 'a factor by which the tumour incidence caused by a specific dose of a chemical carcinogen at low dose rates is multiplied to derive the tumour incidence at high dose rates'. The database used to derive the DRCF is, actually, rather poor. Nonetheless, it was concluded that a DRCF value of 10 is appropriate as a 'default' value if no or insufficient data are available to estimate a compound-specific value of the DRCF. It should be realized that this is a "worst-case" approach of the problem.
In this report no correction for "less than chronic exposure" has been made. The reason for this is that the cancer risks as calculated in this report are mainly determined by the amount of amines which are part of an azo dye, and not the amount of "free"amines, in garment and footwear. As mentioned in Appendix 1 the exposure to the former amines does not have the peak characteristics mentioned above.

Appendix 3 Comparison of the LGC and the RIVM-method (LGC, 1998)

Risk assessment for the exposure of azo dyes from textile: The RIVM method and the LGC method compared

Recently the European Committee Directorate III assigned the "The Laboratory of the Government Chemist" (LGC) to perform a risk assessment on the exposure to azo dyes from textile (LGC, 1998). This risk assessment, whose principle is shown in Fig. 1 of the main text, results in a calculated amount of a carcinogenic amine which may be taken up from textile which contains an azo dye. This amount is then compared with the exposure level which is associated with an (extra) cancer risk of 10^{-6} after lifelong exposure to the amine.

For two pieces of textile the risk assessment as performed by LGC is compared with the risk assessment as performed by RIVM.

Product: Underwear

Product weight: 76 g; Amount of benzidine containing azo dye per gram of product: 3138 µg benzidine.

RIVM calculation:

calculated uptake 285 ng benzidine/day (zie Table 1)
calculated cancer risk: 950×10^{-6} (life-time)

LGC calculation: (see also annex 4 of LGC report)

Exposure $A_{der}^* = A_{der,\,pot}\, m_f . t$
$= (w.wf.\, S_{der})\, m_f . t$

with w the weight of the product per square meter (g/m^2), wf weight fraction of the dye in the product, S_{der} the area of the product which comes into contact with the product (m^2), mf the migration rate of the dye from the product to the skin (fraction per hour), t the duration of exposure (hours), A_{der} the potential amount of the dye to which the skin may be exposed and A_{der}^* the amount of the dye which, daily, may reach the skin.
For underwear A_{der} was experimentally determined as 76 x 3138 µg. Together with an assumed migration fraction of 0.0001/hr and an exposure duration of 10 hrs. A_{der}^* was calculated as:

$$A_{der}^* = 76 \times 3138 \times 0{,}0001 \times 10$$
$$= 238\ \mu g/day$$

Given a maximal uptake of the dye through the skin of 1% the gives the amount of dye taken up as : $E_{eff} = 238 \times 0.01 = 2.38$ µg/dag. Then, assuming 30 % reduction of the dye into benzidine gives for the amount of benzidine taken up: $E_{eff\,(benzidine)} = 2.38 \times 0.3 = 0.71$ µg/dag = 710 ng/dag

Comparing this uptake with the exposure level which is associated with an (extra) cancer risk of 10^{-6} after lifelong exposure to benzidine of 0.3 ng per person per day gives an (excess) cancer risk of 2400×10^{-6} (lifetime)

Similarly,

Product: Blouse

Product weight: 130 g; Amount of *o*-tolidine containing azo dye per gram of the product: 2796 µg *o*-tolidine.

RIVM calculation: calculated uptake 119 ng *o*-tolidine/dag (zie Tabel 1)
calculated cancer risk: 397×10^{-6} (lifetime)

LGC calculation:

Exposure $A_{der}* = (130 \times 2796) \times 0{,}0001 \times 10$
$= 363$ µg/day

Maximal 1 % penetration: $E_{eff} = 363 \times 0.01 = 3.63$ µg/day

30 % azo-reduction:
$E_{eff\,(o\text{-tolidine})} = 3.63 \times 0.3 = 1.09$ µg/day
$= 1090$ ng/day

Excess cancer risk: 3600×10^{-6} (life-time)

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