Acrylamide

Evaluation of the effects on reproduction, recommendation for classification

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Staatssecretaris Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies 'Acrylamide'
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U 1459/AvdB/RA/543-I6
Bijlagen : 1
Datum : 25 november 2002

Mijnheer de staatssecretaris,

Bij brief van 3 december 1993, nr DGV/MBO/U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om naast het afleiden van gezondheidskundige advieswaarden ook te adviseren over andere onderwerpen ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In 1995 heeft de Staatssecretaris van Sociale Zaken en Werkgelegenheid besloten tot het opstellen van een zogenaamde niet-limitatieve lijst van voor de voortplanting vergiftige stoffen. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1, 2 en 3 wat betreft effecten op de voortplanting en stoffen die schadelijk kunnen zijn voor het nageslacht via de borstvoeding. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In dit kader bied ik u hierbij een advies aan over acrylamide. Dit advies is opgesteld door de Commissie Reprotoxische stoffen van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik wil u er op wijzen dat de commissie adviseert acrylamide wat betreft de effecten op de fertiliteit in categorie 2 te classificeren. De commissie is van mening is dat de effecten op de fertiliteit en die op de hersenen en het zenuwstelsel parallelle effecten zijn die wellicht via een zelfde mechanisme tot stand komen. Tevens concludeert de commissie dat het geconstateerde effect op de implantatie een gevolg is van de genotoxische eigenschappen van acrylamide. Het advies van de commissie wijkt af van het standpunt van de Europese Commissie, die acrylamide in categorie 3 heeft geclassificeerd. De Europese Commissie is namelijk van mening dat de effecten op de fertiliteit niet onafhankelijk zijn van die op de hersenen en het zenuwstelsel. Dit verschil van inzicht heeft echter geen gevolgen voor het opnemen van acrylamide op de hierboven genoemde lijst van voor de voortplanting vergiftige stoffen.

Ik heb deze publicatie heden ter kennisname aan de Minister van Volksgezondheid, Welzijn en Sport en aan de Minister van de Volkshuisvesting, Ruimtelijke Ordening en Milieu gestuurd.

Hoogachtend,

prof. dr JA Knottnerus

Bezoekadres
Parnassusplein 5
2511 VX Den Haag
Telefoon (070) 3407520
E-mail: A.vd.burght@gr.nl

Postadres
Postbus 16052
2500 BB Den Haag
Telefax (070) 340 75 23
www.gr.nl

Acrylamide

Evaluation of the effects on reproduction, recommendation for classification

Committee for compounds toxic to reproduction
A Committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

Nr 2002/16OSH, The Hague, 25 November 2002

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Preservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

Preferred citation:
Health Council of the Netherlands: Committee for Compounds toxic to reproduction. Acrylamide; Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2002; publication no. 2002/16OSH.

ISBN: 90-5549-455-0

Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondsheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductietoxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie acrylamide onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit adviseert de commissie acrylamide in categorie 2 (*stoffen die dienen te worden beschouwd alsof zij bij de mens de vruchtbaarheid schaden*) te classificeren en met R60 (*Kan de vruchtbaarheid schaden*) te kenmerken.
- Voor ontwikkelingsstoornissen meent de commissie dat er onvoldoende gegevens beschikbaar zijn. Zij adviseert daarom om acrylamide niet te classificeren.
- Voor effecten tijdens lactatie adviseert de commissie om acrylamide niet te kenmerken wegens onvoldoende gegevens.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends to classify compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed acrylamide.

The committee's recommendations are

- For effects on fertility, the committee recommends to classify acrylamide in category 2 (*substances which should be regarded as if they impair fertility in humans*) and to label acrylamide with R60 (*May impair fertility*).
- For developmental toxicity, the committee is of the opinion that a lack of appropriate data precludes the assessment of acrylamide. Therefore, the committee recommends no classification of acrylamide.
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data acrylamide should not be labelled.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds Toxic to Reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1, 2 or 3) or labelled as may cause harm to breastfed babies (R64).

1.2 Committee and procedure

The present document contains the classification of acrylamide by the Health Council's Committee for Compounds Toxic to Reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by Mrs ir DH Waalkens-Berendsen at the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse

effects with respect to fertility and development and lactation of the above mentioned compound.

Classification and labelling was performed according to the guidelines of the European Union listed in Annex C.

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In 1999, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, the committee has agreed upon a number of additional considerations.

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).

- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration is above an exposure limit for the general population, eg the acceptable daily intake (ADI).

* Organisation for Economic Cooperation and Development

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 1995. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1999. The results of this search were no reason for the committee to adjust the recommendations.

The committee chose to describe human studies in the text, starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

Animal data are described in the text and summarised in Annex D.

1.6 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- Lack of appropriate data preclude assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxic to reproduction is indicated.

1.7 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter 2

Acrylamide

2.1 Introduction

Name	:	acrylamide (CH_2=CHCONH$_2$)
CAS-no.	:	79-06-1
Use	:	improvement of aqueous solubility, adhesion and cross-linking of polymers; flocculant for wastewater treatment, soil stabilization; additive for oil well drilling fluids, papermaking, textiles, paints and cements, gels for biotechnical laboratories
Mol weight	:	71.08
Chem formula	:	C_3H_5NO

Acrylamide is considered to be a genotoxic carcinogen (IAR94). The European Commission has classified acrylamide for effects on fertility in category 3. For effects on development, acrylamide has not been classified.

2.2 Human studies

Fertility

No publications were found concerning effects of acrylamide on human fertility.

Developmental toxicity

No publications were found concerning developmental effects of acrylamide in man.

Lactation

No publications were found concerning the excretion of acrylamide in human breast milk.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarize the fertility and developmental toxicity studies with acrylamide in experimental animals.

Fertility studies

The toxicity of acrylamide on the reproduction of both male and female hooded Long-Evans rats was evaluated in 2 separate studies in which acrylamide was dosed in drinking water. Sexually experienced young adult male rats were offered levels of 0, 50, 100 or 200 ppm (mg/kg water) acrylamide in drinking water for an 11-week period. Exposure to 200 ppm (mg/kg water) resulted in severe hindlimb splaying and mortality, and these male rats were sacrificed at the end of week 6. Exposure to 100 ppm (mg/kg water) resulted in less severe hindlimb splaying, observed from week 8 onwards. Weight gain was severely reduced in the 200 ppm (mg/kg water) group, and less, but consistently, in the 100 ppm (mg/kg water) group. Copulatory behaviour, monitored 1 week before exposure and twice weekly thereafter, showed an increase in the number of mounts and intromissions, slightly in the 25 and 50 ppm (mg/kg water) groups, and statistically significant in the 100 and 200 ppm (mg/kg water) groups. Ejaculated sperm and fertility data, generated 1 week before exposure and in week 9 using untreated females, showed in the 100 ppm (mg/kg water) group a reduction in sperm count and sperm motility, a decrease in number of pregnancies and an increase in post-implantation loss. No treatment-related effects were observed upon necropsy in the males of the 50 and 100 ppm (mg/kg water) groups (Zen86).

Young adult female rats were offered 0, 25, 50 or 100 ppm (mg/kg water) acrylamide in drinking water during a period including 2 weeks premating, mating with untreated male rats, gestation and lactation. Hindlimb-splaying was observed in the female rats of the 100 ppm group (mg/kg water) during week 1-2 of gestation. Fluid intake and body weights of the 100 ppm group (mg/kg water) were consistently reduced. Mating performance and pregnancy rate were unaffected; litter size was

slightly decreased in the 100 ppm (mg/kg water) group. Pup body weights were severely reduced in the 100 ppm (mg/kg water) group and slightly in the 25 and 50 ppm (mg/kg water) groups, but survival at weaning was similar to the control group. Vaginal opening was delayed in the female pups of the 100 ppm (mg/kg water) group (Zen86).

Groups of male hybrid mice (C3H x 101) were injected intraperitoneally either once with 125 mg acrylamide/kg body weight or 5 times with 50 mg acrylamide/kg body weight/day. Subsequent mating to untreated female mice showed a significant increase in post-implantation loss in the female mice mated 4 to 11 days after male exposure. Effects on late spermatids and early spermatozoa were suggested. In a toxicity study at 150 mg/kg four out of 12 animals died (She86).

A group of 50 male rats was orally gavaged with 0 or 30 mg acrylamide/kg body weight per day for 5 consecutive days. Starting day 1 after exposure, each male was caged with a female rat and was given a new virgin female rat every week for 10 weeks. The female rats were killed 13 days after the end of their mating period. The number of mated female rats was slightly reduced in week 1 and 2; the number of corpora lutea was not affected. Post-implantation loss was increased in week 1, 2 and 3; pre-implantation loss was increased in week 1 to 4. No male died during the exposure or breeding period. Body weights in the acrylamide-treated group remained below control values until the end of the study, except during post-exposure week 3 and 7 (Wor87).

Groups of 10-15 young adult male hooded Long-Evans rats were orally gavaged with 0 to 60 mg acrylamide/kg body weight per day for 5 consecutive days. They were mated with untreated female rats 1 to 10 weeks after exposure. No effects were found on the mating index; fertility rate and pre-implantation loss were depressed at week 1 in all dose groups, and in the higher dose groups also in the following weeks. Copulatory behaviour was not affected in any group. Sperm transport in the reproductive tract was decreased in female rats mated with male rats of the higher dose groups. Other parameters of sperm motility (curvilinear velocity, linearity and straight line velocity) were also decreased. Fertilization rate was significantly reduced in female rats mated to male rats of the higher dose groups; these effects recovered in the course of weeks. In the present study full recovery of impaired motor behaviour was observed 7 days after dosing (Sub89).

Costa *et al*. (1992) studied the neurotoxicity of acrylamide (25 and 50 mg/kg body weight) and glycidamide (50 and 100 mg/kg body weight, a metabolite of acrylamide) in male rats after intraperitoneal injection (Cos92). All rats were injected daily for 8 days and sacrificed on day 9. In the reproductive study, sexually mature male rats were injected intraperitoneally with 50 mg/kg body weight acrylamide or glycidamide for 7 days. In the 50 mg acrylamide/kg group, body weight and rotarod performance were

significantly decreased and hindlimb splay was impaired in both acrylamide treated groups. Body weight was decreased in both glycidamide groups; rotarod performance was impaired at 100 mg glycidamide/kg body weight. In the study for reproductive toxicity the only effect of acrylamide at 50 mg/kg was a decreased number of epididymal sperm; this effect was also observed in the 50 and 100 mg/kg body weight glycidamide groups. Testis and epididymis weight, protein/g testis and epididymal sperm cell viability were unaffected after exposure to acrylamide; 100 mg glycidamide/kg body weight decreased these parameters for reproductive effects.

Chapin *et al*. (1995) studied the effects of acrylamide in mice using the continuous breeding protocol. Male and female mice were provided with drinking water containing 0, 1, 10 and 30 ppm (mg/kg water) acrylamide during and after a 14 week cohabitation period (Cha95). The last litter was reared and dosed after weaning until mating at 74 $\pm$ 10 days of age with the same level of compound given to the parents. Neurotoxicity was assessed at several times in both generations by measuring forelimb and hindlimb grip strength. In the F0-generation 30 ppm acrylamide caused a 11% decrease in pup number. Absolute grip strength over the time was altered in the 30 ppm (mg/kg water) acrylamide group for male forelimbs and hindlimbs and for female forelimbs. Female fertility was not affected. When treated F_1-female mice of the high dose group were mated with naive males, no effects were observed on the number of pups delivered per litter. This is in contrast with the mating of treated male mice with naive female mice, were a decreased number of pups was observed. In the male dominant lethal test, the 30 ppm group showed an increase in the number of early resorptions.

Tyl *et al*. 2000 exposed F0 weanling rats to acrylamide in drinking water at 0, 0.5, 2 and 5 mg/kg body weight/day for 10 weeks premating (Tyl00a). F0 females were exposed through gestation and lactation of F1 litters. F0 males, after F0-mating, were removed from exposure and mated for the dominant lethal assay with non-exposed females. F1 weanlings were exposed for 11 weeks to the same dose level as their parents and mated to produce F2 offspring. In both generations body weight gains were affected in the 2 and 5 mg/kg group. Some effects (not significant) were observed on head tilt and foot splay in the F0-generation in all treated groups. In the F1-generation only in males of the high dose group a slight increase (significant) was observed in the number of animals with tilted head. In the F0 parents no treatment related effects were observed on reproductive organs and nervous system tissue. In the F1 parents no effects were observed on reproductive organs and an effect was observed in the males after a special staining in the peripheral nerve in the 5 mg/kg group. In both generations a significant decrease in the number of implantations (F0: 6.8 and 10.4 and F1 11.3 and 6.8 in the control and 5 mg/kg groups, respectively) and number of live pups (F0: 9.8 and 4.5 and F1: 10.8 and 5.1 in the control and 5 mg/kg groups respectively). Furthermore a decreased pup weight was observed in both generations. In the dominant

lethal test, an increased pre- and post implantation loss was observed at 5 mg/kg body weight.

Tyl *et al* 2000 exposed Long-Evans male rats (25/group) to acrylamide in drinking water at 0, 5, 15, 30, 45 or 60 mg/kg bw/day daily for 5 days (days 1 to 5) (Tyl00b). On day 8, males were paired with untreated females, and on day 9 males were evaluated for forelimb and hindlimb grip-strength and necropsied. Sperm-positive females were examined for preimplantation and postimplantation loss at midpregnancy. At 15 to 60 mg/kg bw/day, males exhibit significantly reduced weight gain, reduced mating, fertility and pregnancy indices. At 45 and 60 mg/kg bw/day an increased postimplantation loss was observed and at 60 mg/kg bw/day the sperm beat cross frequency was increased, with no effects on epididymal sperm motility or concentration and a decreased hindlimb grip strength. The authors concluded that effects on fertility and the neurotoxic effects were observed at doses that also resulted in postimplantation loss, possibly by different mecanisms.

General toxicity: neurotoxicity

Edwards *et al* (1977) studied the neurotoxic effects of acrylamide in male Porton rats. After exposure for 14 days on a diet containing 200 ppm acrylamide (10-15 mg/kg bw/day), an increase was found in the landing foot-spread.

Crofton *et al*. (1996) studied the neurotoxicity of acrylamide after acute, 10-day, 30-day and 90-day intraperitoneal exposure and found overall NOELs (LOELs) of 37.5 (75), 7.5 (30), 5(10) and 3.3 (3.3) mg/kg body weight, respectively (Cro96). The parameters tested for neurotoxicity were grip strength, horizontal activity, vertical activity, startle response, sciatic pathology and spinal pathology.

Developmental toxicity

Young adult female rats (Long Evans hooded rats) were offered 0, 25, 50 or 100 ppm (mg/kg water) acrylamide in drinking water during a 2-week premating period, (mating with untreated males), gestation and lactation. Hindlimb-splaying was observed in the female rats of the 100 ppm (mg/kg water) group during week 1-2 of gestation. Fluid intake and body weights of the 100 ppm (mg/kg water) group were consistently reduced. Mating performance and pregnancy rate were unaffected; litter size was slightly decreased in the 100 ppm (mg/kg water) group. Pup body weights were severely reduced in the 100 ppm (mg/kg water) group and slightly in the 25 and 50 ppm (mg/kg water) groups, but survival at weaning was similar to the control group. Vaginal opening was delayed in the female pups of the 100 ppm (mg/kg water) group (Zen86).

Sexually mature T-stock female mice were mated to HT-stock male mice and were used for the evaluation of teratogenic effects as part of a spot test. The females received an intraperitoneal injection of 0, 50 or 75 mg acrylamide/kg body weight either on day 12 of gestation only, or on days 10, 11 and 12 of gestation. The female mice were sacrificed on day 18 of gestation and only litters with 4 pups or more were used for evaluation. The 3x75 mg/kg body weight group showed a reduction in litter size due to post-implantation loss, a high percentage of living foetuses with growth retardation, hypoplasia of lymphatic organs and centres for haematopoiesis in liver and bone marrow, haemorrhages in the placenta and tail malformations. Foetotoxicity was concluded to be mainly directed at mesenchymal tissues. Maternal toxicity was not described (Neu89).

Developmental toxicity was evaluated by gavaging groups of 17 female, pregnant Fisher 344 rats with daily doses of 20 mg acrylamide/kg body weight dissolved in deionized water or with vehicle control from days 7 to 17 of gestation. Upon birth the pups were cross-fostered. No effects were observed on body weight or general health of either treated parents or pups, or on litter size. On postnatal days 14 and 21, male and female pups were sacrificed for analysis of dopamine receptor binding in the brain. Dopamine receptor binding was decreased in 2-week old male pups exposed *in utero* and fostered by either exposed or control dams and in 2-week old female control pups fostered by exposed dams. No effects on dopamine levels were measured in 3-week old pups (Agr81).

Groups of 26 pregnant Sprague-Dawley rats were gavaged with 0, 2.5, 7.5 or 15 mg acrylamide/kg body weight from gestation day 6 through 20 when they were sacrificed. Maternal body weight gain was reduced in the 7.5 and 15 mg/kg groups. Very few effects were found upon foetal examination: the percentage of foetuses with extra ribs increased in a dose-related way, but did not reach the level of statistical significance (Fie90).

Groups of 30 pregnant Swiss CD-1 mice were gavaged with 0, 3, 15 or 45 mg acrylamide/kg body weight from gestation day 6 through 17 when they were sacrificed. Maternal body weight gain and gravid uterine weight were reduced in the 15 and 45 mg groups. Few effects were found upon foetal examination: foetal body weight was reduced in the 45 mg group; the percentage of foetuses with extra ribs was increased in a dose-related way, but did not reach the level of statistical significance (Fie90).

Groups of 12 female Sprague Dawley rats [Crl:CD®(SD) BR] received by oral gavage doses of 1, 5, 10, 15 or 20 mg acrylamide/kg body weight from day 6 of gestation up to day 10 of lactation. F0-female rats were monitored for general health and body weight: females of the 15 and 20 mg/kg treatment groups showed a reduced weight gain during gestation and hind limb splaying. In the 10 mg/kg treatment group reduced body weight gain was observed during lactation. Upon sacrifice the uterus was

examined microscopically. Pup mortality increased in the 15 mg/kg treatment group and particularly in the 20 mg/kg treatment group. In the pups of the 5 mg/kg treatment group preweaning body weight was transiently reduced. In the higher dose groups it was permanently reduced, in a dose-related way. Some of the pups were examined for brain weight (postnatal day 11); a slight reduction in absolute brain weight, and an increase in relative brain weight was found. Of these pups, brain, spinal cord and peripheral nerve were examined microscopically. Other pups were tested for open-field motor activity (postnatal days 13, 17, 21, 59), auditory startle behaviour (postnatal day 22) and passive avoidance (postnatal days 24, 59); the 15 mg group was the highest dose group tested. Only slight to no behavioural effects were found (Wis95).

Lactation

Husain *et al*. (1987) studied the neurotoxicity of acrylamide in the developing rat brain (Hus87). Acrylamide (25 mg/kg body weight) was administered orally to mothers daily during the lactation period. Pups were sacrificed on PN day 2, 4, 8, 15, 30, 60 and 90. Noradrenaline, dopamine and 5-hydroxytryptamine levels were decreased in the brain of pups which were exposed via the lactating mother to acrylamide.

Friedman *et al*. (1999) duplicated the above mentioned study (Hus87) and demonstrated that acrylamide induced toxicity in the mothers (mortality, severe reduced feed and water consumption, decreased body weight and body weight gain and behavioural neurotoxicity) at 25 mg/kg body weight. Nursing offspring exposed via the mother to acrylamide exhibited increased mortality and reduced body weight associated with little or no milk in stomach. On PN day 91 F1-males did not demonstrate a difference in grip strength.

2.4 Overall conclusion

No data were available concerning effects of acrylamide on human fertility.

Reduction in fertilization rate was observed in female rats mated with acrylamide treated male rats (Sub89). In addition, acrylamide induced dominant lethal effects (post-implantation loss) (She86, Wor87, Zen86, Cha95, Tyl00a/b). Acrylamide has adverse effects on male and female reproduction capacity (increase in number of mounts and intromissions, reduced sperm count, sperm motility, number of pregnancies) in animals, but these effects were predominantly found at levels at which neurotoxic effects were observed (hind limb splaying) as well (Zen86, Cha95). The committee is of the opinion that the effects on fertility can not be explained from the neurotoxic effects and both effects may share a common molecular mecanism (via motorproteins) (Cos92, Cro96, Tyl00b). In addition, one of the observed effects,

implantation loss, is likely caused by the genotox properties of acrylamide (and not due to neurotoxicity). In conclusion, in view of the animal data with respect to the specific effects on fertility, the committee recommends to classify acrylamide in category 2 (substances which should be regarded as if they impair fertility in humans) and to label the substance with R60 (May impair fertility).

No data were available concerning developmental effects of acrylamide in man.

Effects on embryonic, foetal and postnatal development were observed. These effects include reduced body weight (Fie90; Zen86) and transient effects as reduced dopamine levels in the brain, reduced open-field activity and auditory startle habituation (Agr81; Wis95). However, either maternal toxicity was not described correctly or the foetal effects occurred only at maternally toxic levels. Although it may be expected on the basis of its genotoxic mechanism of action that acrylamide would influence the development of the foetuses, it can not be excluded that the effects observed in the offspring were secondary to maternal toxicity.

In conclusion, a lack of appropriate data precludes the assessment of acrylamide for developmental effects. Therefore the committee recommends not to classify acrylamide with respect to developmental effects.

Husain *et al*. (1987) found effects on the developing brain of pups nursed by mothers exposed orally to 25 mg acrylamide/kg body weight/day. Friedman *et al*. (1999) duplicated this study and demonstrated that this dose induced generalized toxicity in dams and pups. For this reason, the effects found on the developing brain in the study of Husain *et al*. (1987) were not considered specific. No other studies on effects on lactation were available. Therefore, a lack of appropriate data precludes the assessment of acrylamide for labelling for effects during lactation.

Proposed classification for fertility

Category 2, R 60.

Proposed classification for developmental toxicity

A lack of appropriate data precludes classification of acrylamide for developmental effects.

Proposed labelling for effects during lactation

Lack of appropriate data precludes assessment of acrylamide for labelling for effects during lactation.

References

Agr81 Agrawal AK, Squibb RE. Effects of acrylamide given during gestation on dopamine receptor binding in rat pups. Toxicol. Lett. 1981; 7: 233-237.

Cha95 Chapin RE, Fail PA, George JD ao. The reproductive and neural toxicities of acrylamide and three analogues in Swiss Mice, evaluated using the continuous breeding protocol. Fund. Appl. Toxicol. 1995; 27: 9-24.

Cos92 Costa LG, Deng H, Gregotti C, Manzo, L, Faustman EM, Bergmark E, Calleman CJ. Comparative Studies on the Neuro- and Reproductive Toxicity of Acrylamide and its Epoxide Metabolite Glycimide in the Rat. NeuroToxicol. 1992;13: 219-224.

Cro96 Crofton KM, Padilla S, Tilson HA, Anthony DC, Raymer JH, MacPhail RC. The impact of Dose Rate on the neurotoxicity of acrylamide: The Interaction of Administered Dose, Target Tissue concentrations, tissue damage, and functional Effects. Toxicol. Appl. Pharmacol. 1996; 139: 163-176.

Edw77 Edwards PM and Parker VH, A Simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats, Tocicol. Appl. Pharmacol. 1977: 40, 589-591.

Fie90 Field EA, Price CJ, Sleet RB, Marr MC, Schwetz BA, Morrissey RE. Developmental toxicity evaluation of acrylamide in rats and mice. Fundam. Appl. Toxicol. 1990; 14: 502-512.

Fri99 Friedman MA, Tyl RW, Marr MC, Myers CB, Gerling FS, Ross WP. Effects of lactational administration of acrylamide on rats and offspring. Reprod. Toxicol. 1999; 13: 511-520.

Hus87 Husain R, Dixit R, Das M, Seth P. Neurotoxicity of Acrylamide in Developning Brain: change in the levels of brain biogenic amines and activities of monoamine oxidase and acetylcholine esterase. Ind. Health. 1987; 25: 19-28.

IAR94 IARC International Agency for Research on Cancer. Acrylamide in some industrial chemicals. Lyon France IARC 1994; 389-433, 502,527. IARC monograph on the evaluation of carcinogenic risk to humans vol 60.

Neu90 Neuhäuser-Klaus A, Schmahl W. Mutagenic and teratogenic effects of acrylamide in the mammalian spot test. Mutation Res. 1990; 226: 157-162.

She86 Shelby M, Cain K, Hughes L, Braden P, Generoso W. Dominant lethal effects of acrylamide in male mice. Mutation Res. 1986; 172: 35-40.

Sub89 Sublet VH, Zenick H, Smith MK. Factors associated with reduced fertility and implantation rates in females mated to acrylamide-treated rats. Toxicology 1989; 55: 53-67.

Tyl00a Tyl RW, Friedman MA, Losco PE, Fisher LC, Johnson KA, Strother DE, Wolf CH. Rat two-generation reproduction and dominant lethal study of acrylamide in drinking water. Reprod. Toxicol. 2000; 14: 385-401.

Tyl00b Tyl RW, Marr MC, Myers CB, Ross WP and Friedman MA. Relationship between acrylamide, reproductive and neurotoxicity in male rats. Reprod. Toxicol. 2000; 14: 147-157.

Tox95 Niesink RJM, de Vries J, Hollinger MA, eds, Toxicology, Principles and Applications, Boca Raton: CRC Press, 1995:385

Wis95 Wise LD, Gordon LR, Soper KA, Duchai DM, Morrissey RE. Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. Neurotoxicol. Teratol. 1995; 17: 189-198.

Wor87 Working PK, Bentley KS, Hurtt ME, Mohr KL. Comparison of the dominant lethal effects of acrylonitrile and acrylamide in male Fisher 344 rats. Mutagenesis 1987; 2: 215-220.

Zen86 Zenick H, Hope E, Smith MK. Reproductive toxicity associated with acrylamide treatment in male and female rats. J. Toxicol. Environ. Health 1986; 17: 457-47.

Literature used but not cited

Adl93 Adler I-D, Zouh R, Schmid E. Pertubation of cell division by acrylamide in vitro and in vivo. Mutation Res. 1993; 301: 249-254.

Adl94 Adler I-D, Reitmeir P, Schmöller, Schriever-Schwemmer G. Dose response for heritable translocations induced by acrylamide in spermatids of mice. Mutation Res. 1994; 309:285-291.

Dea88 Dearfield KL, Abernathy CO, Ottley MS, Brantner JH, Hayes PF. Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutation Res. 1988; 195: 45-77.

Hei97 Heindel J, Fail PA, George J, Grizzle TB. Acrylamide. Environ Health Perspec. 1997; 105 (suppl. 1): 259-260.

Kha92 Khanna VK, Husain R, Seth PK. Protein malnourishment a predisposing factor in acrylamide toxicity in pregnant rats. J. Toxicol. Environ. Health 1992; 36: 293-305.

See91 Seely KA, Aggeler J. Modulation of milk protein synthesis through alteration of the cytoskeleton in mouse mammary epithelial cells cultured on a reconstituted basement membrane. J. Cell. Physiology 1991; 146: 117-130.

Sic92 Sickles DW. Toxic neurofilamentous axonopathies and fast anterograde axonal transport. IV. In vitro analysis of transport following acrylamide and 2,5-hexanedione. Tox. Lett. 1992; 61: 199-204.

Sic95 Sickles DW, Welter DA. Acrylamide arrests mitosis and prevents chromosome migration in the absence of changes in spindle microtubules. J. Environ. Health 1995; 44: 73-86.

Sic96 Sickles DW, Brady, ST, Testino A ao. Direct effect of the neurotoxicant acrylamide on kinesin-based microtubule motility. J. Neu. Res. 1996; 46: 7-17.

Vog93 Vogel E, Nivard MJM. Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutation Res. 1993; 8 (1) 57-81.

Wal81 Walden R, Squibb RE, Schiller CM. Effects of prenatal and lactational exposure to acrylamide on the development of intestinal enzymes in the rat. Toxicol. Appl. Pharmacol. 1981; 58: 363-369.

WHO85 WHO Environmental Health Criteria 49: Acrylamide. World Health Organization, Geneva.

A The committee

B Comments on the public draft

C Directive (93/21/EEG) of the European Community

D Fertility and developmental toxicity studies

E Abbreviations

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist; Institute for Risk Assessment Sciences, Utrecht
- JN van den Anker
  Professor of pediatrics and pharmacology; The George Washington University Medical Center, USA
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, Den Haag
- HFP Joosten
  Toxicologist; NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  Professor of medical genetics, paediatrician; UMC, Utrecht
- JHJ Copius Peereboom-Stegeman
  Toxicologist; Catholic University Nijmegen, Nijmegen
- AH Piersma
  Reproductive toxicologist; National Institute of Public Health and the Environment, Bilthoven
- N Roeleveld
  Epidemiologist; Catholic University Nijmegen, Nijmegen.
- DH Waalkens-Berendsen
  Reproductive toxicologist; TNO Nutrition and Food Research, Zeist
- PJJM Weterings
  Toxicologist; Weterings Consultancy BV, Rosmalen

- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present document was prepared by DH Waalkens-Berendsen, from the TNO Nutrition and Food Research in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: A Aksel
Lay-out: J van Kan, M Javanmardi

Annex **B**

Comments on the public draft

A draft of the present report was released in 1999 for public review. The folowing persons and organisations have commented on the draft document:

- P Ungeheur,
  Acrylamide Producers Associations Europe (AMPA) Germany

Annex **C**

Directive (93/21/EEC) of the European Community

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:

T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:

T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:

Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1) *Effects on male or female fertility*, includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would

interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2) *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peripostnatal defects, and impaired postnatalmental or physical development up to and including normal pubertal development.

Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity.

The placing of a compound in Category 1 for effects on Fertility and/or Developmental Toxicity is done on the basis of epidemiological data. Placing into Categories 2 or 3 is done primarily on the basis of animal data. Data from *in vitro* studies, or studies on avian eggs, are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

In common with most other types of toxic effect, substances demonstrating reproductive toxicity will be expected to have a threshold below which adverse effects would not be demonstrated. Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administrated, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in Category 3, or even no classification, will be warranted.

Annex V of the Directive specifies a limit test in the case of substances of low toxicity. If a dose level of at least 1000 mg/kg orally produces no evidence of effects toxic to reproduction, studies at other dose levels may not be considered necessary. If data are available from studies carried out with doses higher than the above limit dose, this data must be evaluated together with other relevant data. Under normal circumstances it is considered that effects seen only at doses in excess of the limit dose would not necessarily lead to classification as Toxic to Reproduction.

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would

lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification into Category 2 should only be made where there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it was demonstrated that impaired fertility in animal studies was due to failure to mate, then for classification into Category 2, it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposue is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as 'Toxic to Reproduction', unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

a) toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or
b) on the basis of results of one or two generation studies in animals which in- dicate the presence of adverse effects on the offspring due to transfer in the milk, and/or
c) on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

Table 1.1 Fertility studies with acrylamide.

authors	species	route	experimental period	dose	findings	rem.
Zenick *et al*. (1986)	Long-Evans hooded rat (males)	drinking water	70 d and onwards until treated for 11 weeks	0, 50, 100, 200 ppm (mg/kg water)	*200 ppm* group: severe toxicity and mortality w 5 *100 ppm* group: hind limb splaying, reduced body weight; stat. sign. increase of sexual activity, reduced ejaculatory ability and sperm counts; decrease in pregnancies, increase in post-implantation loss *50 ppm* group: increased sexual activity	
idem	Long-Evans hooded rat (females)	drinking water	administration: 2 w premating, mating, gestation, lactation sacrifice offspring: PN d 42	0, 25, 50, 100 ppm (mg/kg water)	*100 ppm* group: gestation w 1+2 hindlimb splaying. Reduced fluid intake and body weight. Reduced pup body weight, delayed vaginal opening *50 ppm* group: reduced body weight during lactation. Reduced pup body weight *25 ppm* group: reduced body weight d 7, 14	
Shelby *et al*. (1986)	hybrid (C3H x 101) mice (males)	i.p. injection	administration: 1x, or 5x daily mating: 1 d after exposure	0 and 125 and 50 mg/kg bw, respectively	4-11 d after exposure: increase post-implantation loss Effects on late spermatids, early spermatozoa	
Working *et al*. (1987)	Fisher 344 rats (males)	oral gavage	administration: 5x, daily mating: 10 weeks (1 female/w)	0 and 30 mg/kg bw	Males decreased body weights; except during post-exposure weeks 3 and 7. up to 4 weeks: increased pre-implantation loss up to 3 weeks:increased post-implantation loss	

Table 1.2 Fertility studies with acrylamide.

authors	species	route	experimental period	dose	findings	remarks
Sublet *et al*. (1989)	Long-Evans hooded rats (males)	oral gavage	administration: 5x, daily mating: 1-10 w after exposure	0, 5, 15, 30, 45, 60 mg/kg bw	*All groups* w 1: decrease entry of sperm to uterus; fertility rate, increase pre-implantation loss *45 mg group* w 2+3: decrease in sperm motility; *all groups*: increased pre- and post-implantation loss	general toxicity not described in detail; 7 days after dosing full recovery of impaired motor behaviour was observed.
Costa *et al*. (1992)	Male Sprague Dawley rats	ip	Neurotoxicity study: injected once a day for 8 days; Sacrifice 24 hours after last dose. Study for reproductive parameters: injected once a day for 7 days. Sacrifice 24 hours after last dose.	25 and 50 mg/kg bw 50 mg/kg bw	50 mg: decreased bw and impaired rotarod performance 25 and 50 mg: impaired hindlimb splay 50 mg: decreased number of epididymal sperm	
Chapin *et al*. (1995)	Swiss mice (males and females)	drinking water	1. continuous breeding 2. mating exposed F1-generation with naive males 3. mating exposed F1-generation with naive females	0, 3, 10, 30 ppm (mg/kg water)	1. 30 ppm :11 % decrease in pup number 2. 30 ppm: decreased number of pups/litter 3. 30 ppm: increased number of early resorptions	effects on grip strength
Tyl *et al*. (2000a)	Fischer 344 rats	drinking water	F0/F1: males, 10 weeks premating, F0/F1 females premating, gestation and lactation in the 2-generation test. F0 males in the dominant lethal test 10 weeks premating	0, 0.5, 2 and 5 mg/kg bw	F0/F1: 2 and 5 mg/kg decreased body weight; F0: some not significant effects on neurotoxicity; F1: slight significant increase neurotoxicity (5 mg) F0/F1: histopahology, no effect on reproductive organs; F0: nervous system tissue no effect; F1: effect on nervous tissue after special staining (5 mg) F0/F1: significant decrease in the number of implantations and live pups, decreased pup weight (5 mg) Dominant lethal test: increase pre/post implantation loss.	

Table 1.2 Continued.

authors	species	route	experimental period	dose	findings	remarks
Tyl *et al*. (2000b)	long Evans male rats (25/ group)	gavage via water	administration daily for 5 days. Mating on day 8 with untreated females. Day 9, males were evaluated for hind-, forlimb grip strength and necropsied.	0, 5, 15, 30, 45, 60 mg/kg/ day	15 to 60 mg/kg: reduced weight gain males, reduced mating, fertility, pregnancy indices. 45 and 60 mg/kg: increased post-implantation loss and dominant lethal factor. 60 mg/kg/day: increased sperm beat cross frequency, no effect on sperm motility, decreased hindlimb grip strength.	

bw = body weight; d = day; i.p. = intraperitoneal; PN = postnatal; w = week

Table 2.1 Developmental toxicity studies with acrylamide.

authors	species	route	experimental period	dose	findings	remarks
Zenick *et al*. (1986)	Long-Evans hooded rat (females)	drinking water	administration: 2 w premating, mating, gestation, lactation sacrifice offspring: PN d 42	0, 25, 50, 100 ppm (mg/kg water)	*100 ppm* group: gestation w 1+2 hindlimb splaying. Reduced fluid intake and body weight. Reduced pup body weight, delayed vaginal opening *50 ppm* group: reduced body weight during lactation. Reduced pup body weight *25 ppm*: reduced body weight d 7, 14	
Neuhäuser -Klaus & Schmahl (1989)	T stock mouse (females) and HT mouse (males)	i.p. injection	administration: gestation d 12 (high dose group) or d 10, 11 and 12 (all groups) sacrifice: gestation d 18	0, 50, 75 mg/kg bw	*all dose groups*: increased incidence of kinked tail and haemorrhages *75 mg/kg*, 3x dosed group: increased post-implantation loss, decreased litter size and foetal weight; hypoplasia lymphatic organs and centres of haematopoiesis in liver and bone marrow	Maternal toxicity was not described
Field *et al*. (1990)	Sprague-Dawley rat	oral gavage	administration: gestation d 6-20 sacrifice: gestation d 20	0, 2.5, 7.5, 15 mg/kg bw	7.5 and 15 mg/kg bw groups: reduced maternal weight	
idem	Swiss CD-1 mouse	oral gavage	administration: gestation d 6-17 sacrifice: gestation d 17	0, 3, 15, 45 mg/kg bw	*15 and 45 mg/kg bw* group: reduced maternal weight gain and gravid uterus weight; *45 mg group*: reduction of foetal body weight	

Table 2.2 Developmental toxicity studies with acrylamide.

authors	species	route	experimental period	dose	findings	rem.
Agrawal & Squibb (1981)	Fisher 344 rat	oral gavage	administration: gestation d 7-16 sacrifice: lactation d 14 and 21	20 mg/kg bw/d	PN d 14: control female offspring fostered by exposed dams and male offspring fostered by (un)exposed dams: reduced content of dopamine receptors PN d 21: no effect	
Wise *et al*. (1995)	Sprague-Dawley rat	gavage	administration: gestation d 6-lactation d 10	0, 5, 10, 15, 20 mg/kg/d	15-20 mg: increased pup mortality 10-20 mg: reduced maternal body weight gain, hindlimb splaying 5-20 mg: dose-related decrease in pup bw	

bw = body weight; d = day; i.p. = intraperitoneal; PN = postnatal; w = week

Annex **E**

Abbreviations

Abbreviations used:

bw	=	body weight
d	=	day
F	=	female(s)
i.p.	=	intraperitoneal
i.v.	=	intravenous
M	=	male(s)
n	=	number
NOAEL	=	no adverse effect level
OECD	=	Organisation for Economic Cooperation and Development
PN	=	postnatal