Deoxynivalenol (DON)

Gezondheidsraad **Vice-president**
Health Council of the Netherlands

To the Minister of Health, Welfare and Sport

Subject	: Presentation of advisory report about deoxynivalenol in wheat
Your reference	: GZB/VVB 2022533
Our reference	: 3641/LP/tvdk/669-F
Annexes	: 1
Date	: 23 October 2001

Dear Minister,

Pursuant to your request stated in letter no. GZB/ VVB 2022533, I hereby submit to you an advisory report about deoxynivalenol (DON) in wheat. It has been drafted by a committee formed for that purpose and has been assessed by the Health Council's Standing Committees on Health and Environment, and on Nutrition. In accordance with the request for an advisory report, I have also submitted this advisory report to the State Secretary for Agriculture, Nature Conservation and Fisheries. In accordance with the mission of the Health Council, the committee has limited the scope of the report to the describing the significance for human health of certain levels of exposure to DON.

Yours faithfully,

(signed)
JGAJ Hautvast

P.O Box 16052
NL-2500 BB Den Haag
Telephone +31 (70) 340 75 20
Telefax +31 (70) 340 75 23

Visiting Address
Parnassusplein 5
NL - 2511 VX Den Haag
email: GR@gr.nl

Deoxynivalenol (DON)

to:

the Minister of Public Health, Welfare and Sports

No 2001/23E, The Hague, 23 October 2001

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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.

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Contents

Executive summary

Deoxynivalenol (DON) is a toxin formed by fungi of the genus *Fusarium*. These fungi occur in the soil and can infect cereals. The level of *Fusaria* infections in wheat and the resulting DON content of wheat strongly depend on the weather conditions during the growth and bloom of the cereal and on the agricultural techniques used. DON is a contaminant of wheat products that is not easy to avoid.

In 1998, unfavourable weather conditions in Western Europe led to a high level of *Fusaria* infections in wheat and to correspondingly high DON contents. As a consequence, young children ingested relatively high amounts of DON through wheat products in 1999. This prompted the Minister of Health, Welfare and Sports and the Secretary of State of Agriculture, Nature Management and Fisheries to approach the Health Council for an assessment of the possible health risks of exposure to DON, set off against the importance of wheat for public health. A Committee of the Council prepared this assessment.

In laboratory animals, DON can reduce the rate of weight gain during growth. At higher levels of exposure it can adversely affect the immune system, fertility and the fetus. There are no data that suggest the presence of such effects in men. One must realise, however, that the possible occurrence of such effects has not been investigated in humans.

The Committee was asked to evaluate the significance for human health of current levels of DON intake. The point of departure in this work was the formulation of a

Tolerable Daily Intake (TDI). This is the highest lifelong exposure that may reasonably be believed not to have adverse effects in a certain human population.

In animal studies the first effect to occur with low exposure to DON is a reduced weight gain. The Committee derived TDIs based on studies in this effect in mice and pigs. This results in TDIs of 1.0 and 0.5 microgram per kilogram bodyweight per day, respectively. Customarily the Committee has selected the lower value: 0.5. It should be noted that, if DON at all affects human health, it concerns the rate of weight gain in children.

In a number of cases, the actual levels of exposure to DON are occasionally higher than the TDI of 0.5 microgram per kilogram bodyweight per day. In 1999, a year during which wheat had relatively high DON contents, half of all children aged from one to four years consumed more than 1.3 microgram DON per kilogram bodyweight per day. For one out of twenty children in this age group this figure was higher than 2.4. What is the significance of these facts in terms of health? An answer to this question requires further examination, moving beyond establishment of the fact that the exposure is lower or higher than the TDI. This concerns the following:

- Since the method for deriving a TDI involves the use of uncertainty factors, the value obtained can be described as 'cautious'. As a result, exposures that exceed the TDI but which are limited and of short duration will not necessarily result in adverse effects.
- A slightly reduced weight gain is undesirable but not a cause for concern. Results of animal studies also indicate that the effects of DON can be compensated later on when exposure is lower or absent.
- Vulnerability for any effects on growth or development will be most noticeable when the rate of growth is highest. During this phase (i.e. the first six months of life), children consume little or no wheat and DON intake is thus absent or low.
- When deriving a TDI it is assumed that exposure is continuous. Yet, in laboratory animals, it has been shown that the effects of DON under changing exposure levels (i.e. the actual situation in humans) are less pronounced than in the case of continued exposure.

All things considered, it can be concluded that exposure up to a few times and incidentally higher than 0.5 microgram per kilogram bodyweight per day is very unlikely to inhibit the rate of weight gain. Should this effect actually occur in some children, then it will be very minor and (if exposure is not continuously high) can be compensated later on.

Set of against an effect on weight gain, effects on the immune system, on fertility, and on the fetus are more substantial. In animal studies these occur at higher intakes of DON. On the basis of these effects the resultant TDI is 2.5 to 5 microgram per

kilogram bodyweight per day. This implies that below this level effects on the immune system, on fertility, and on the unborn child can be virtually ruled out. This also applies to a continuous intake over a long period of time, and to an unfavourable year such as 1999.

Nonetheless, the Committee feels that exposure to toxins such as DON should be kept as low as possible. Accordingly, the value of 0.5 microgram per kilogram bodyweight per day can serve as a guideline. Reduction of exposure to DON can be reached to some extent through reduction of the DON content of wheat. The Dutch Commodity Board for Cereals, Seeds and Legumes has made specific proposals for such measures. Since the majority of the wheat consumed in the Netherlands is grown in other European countries, international application of the above-mentioned measures is desirable.

Another approach is the exclusion for human consumption of wheat with a high DON content. A measure in this direction has already been taken in 1999, when the government and the business community agreed a so-called action limit for DON in wheat products of 500 microgram per kilogram. Data on the distribution of the DON content of unprocessed wheat show that if this limit were applied to wheat, it would be sufficiently low to prevent exposure to DON at levels in excess of 1.5 microgram per kilogram bodyweight per day. In order to prevent exposure higher than 0.5 microgram per kilogram bodyweight per day, it would be necessary to set a limit for wheat of 100 microgram per kilogram.

Food stuffs containing wheat are staple foods in the Netherlands, and are a good source of a number of nutrients. The Committee therefore advises against reduction of exposure to DON by decreasing wheat consumption.

Chapter 1

Introduction

1.1 Fusarium and mycotoxins

The presence of mycotoxins in foodstuffs is a world-wide problem (WHO00). The usual processes of food preparation such as heating or preserving have little effect on the concentrations of many toxins. To date, more than 300 mycotoxins have been described. Exposure to such toxins can have adverse effects on the function of the liver, the kidneys and the immune system. At the cellular level, it can also affect the supply of energy and protein synthesis. It can also cause inflammation reactions and organ damage. Furthermore, some toxins, such as the aflatoxins, are carcinogenic.

Cereals such as wheat, barley, rye, oats and maize can become infected by soil fungi. Many such fungi belong to the genus *Fusarium*, two of the most important species being *F. graminearum* and *F. culmorum.* These *Fusaria* can lead to diminished crop yields. They produce several types of toxins, such as trichothecenes, fumonisins and zearalenones. Deoxynivalenol (DON) is a member of the first group.

1.2 Request for advice and Committee

In April 1999, the National Commodity Inspectorate investigated various cereal products, including breakfast cereals, and found DON concentrations of up to about 2000 micrograms per kilogram of product ($\mu g/kg_{product}$). At that time, the 'action limit' was 1000 $\mu g/kg_{product}$. This was sufficient cause for this Inspectorate to put the following questions to RIVM (quoted from a letter dated 9 June 1999, reference

MW/mk/U-99/90986): ‘What is the ‘DON burden’ imposed on the average Dutch consumer via wheat and wheat products? What is its significance, from this point of view, for future statutory standards?’ RIVM (National Institute of Public Health and the Environment) issued its report on this matter in November 1999 (Pie99).

The Minister of Public Health, Welfare and Sport, also on behalf of the State Secretary for Agriculture, Nature and Fisheries, has asked the Health Council for its advice. These members of the government require a ‘circumspect weighing-up (…) of the advantages and possible drawbacks of the consumption of bread and cereal products’. These members of the government stated that ‘This consideration should take into account estimates of exposure, the potential harmfulness of DON at concentrations in excess of the proposed limit, the toxicological evidence in support of this, and the importance of bread and cereal products in the daily diet’. Annex A contains the full text of the request for advice.

On 6 November 2000, Prof. JGAJ Hautvast, vice-president of the Health Council, set up a Committee charged with responding to the above-mentioned request for advice. Annex B gives details of the make-up of this Committee.

Chapter **2**

Aspects of the cultivation of *Fusaria* and DON

2.1 The biology of *Fusaria*

During the winter, *Fusaria* can survive in the soil, sustained by the remains of infected plants. The fungus overwinters either as thick-walled chlamydospores or as hyphae. The fungus itself overwinters in seeds, fragments of cereals and maize, and on weeds of the grass family. *Fusaria* also reproduces by means of sexual and asexual reproduction, using ascospores and conidiospores. Ascospores are mainly found in crop fragments that are left in the field after harvesting, especially fragments of maize. Since ascospores cannot travel very far, their effect is mainly limited to the area of land on which they were created. Conidiospores are mainly formed on the above-ground portions of infected plants. These are dispersed by the impact of raindrops and by wind, thereby infecting adjoining areas of land (GZP00).

2.2 The consequences of *Fusaria* infections for crops

Crops can be contaminated by infected seed, by chlamydospores in the soil or by ascospores formed on fragments of crops. The infection is mainly spread via conidiospores. This initially takes place during the autumn and winter, via the base of the stem and the leaves. The base of the stem turns brown or large brown spots and stripes appear on the lower internodes. When the crop is in bud *Fusaria* enters the plants via the stem, causing the stems to buckle. Severe infestations result in the accelerated ripening of the stalk. Infection of the ear mainly occurs in the spring and

summer, when the plants are in blossom. The route of infection is via the open flowers. Infection leads to a reduction in average grain weight, less flour, poorer-quality dough, a lower nutritional value and higher concentrations of mycotoxins.

2.3 Mycotoxins produced by *Fusaria*

Up to the moment that the crop is harvested, and even thereafter, the spread of the fungus and the amounts of toxin that it produces in infected grains depends on factors such as the level of humidity. Other factors are the exact species of *Fusarium* involved and the degree of stress experienced by the fungus. There is only a limited association between the extent of fungal growth (expressed in terms of quantity of mycelium and number of spores) and the level of toxin production.

The susceptibility of crops to fungal infections is governed by specific factors. One such factor is water, in the form of long periods of wet weather, high relative humidity or even dry periods followed by heavy rain. Other factors are temperature (large differences between daytime and night-time temperatures), insects and plant diseases. The inappropriate use of fertilisers, fungicides, herbicides and pesticides can also exert an effect. Toxin production depends on a variety of factors. It is virtually impossible to predict; it can only be measured after the crop has been harvested.

2.4 DON and other *Fusarium* toxins

Some toxins serve to increase the virulence of the fungus. However, it is seldom clear whether or not they perform a specific function for the fungus or whether they are merely the by-products of its metabolism. DON is a member of the group of toxins known as the tricothecenes, which are characterised by the presence of an epoxide group (12,13-epoxide). Deoxynivalenol is related to nivalenol, which is much less common in nature. Although it often occurs in cereal products in association with other trichothecenes, DON is the most commonly occurring toxin in this group.

2.5 DON in wheat

While *Fusarium* infestations can reduce wheat yields (see 2.2), the presence of DON in wheat has little effect in this regard. However, the inclusion of infected wheat in animal feeds can cause economic damage in the livestock industry, especially in pigs. The damage caused is a direct result of DON's effects, namely a reduced rate of weight gain and feed conversion, and an increased susceptibility to infectious diseases. The possible presence of DON residues in animal products such as meat and milk is not hazardous to humans.

There are increasing reports, especially from northern countries, of the presence of DON in cereals, particularly wheat. Affected countries include Canada, the Scandinavian countries, as well as states in central and eastern Europe. This may result from the increasing tendency of farmers to sew wheat after harvesting maize in the same field, from a failure to completely turn the soil over after harvesting a crop and from the use of wheat varieties with short blades of straw. According to analyses of wheat harvested since 1983, Dutch wheat generally has DON concentrations of 20 to 500 µg/kg (Nij96, Tan90; see also tables 5.1 and 6.2, as well as annex C). 1987 and 1998 are often referred to as 'problem years' (Gar94, Mül99).

2.6 Controlling *Fusaria*

From time to time, severe *Fusarium* infections occur in cereals and cause reduced crop yields. Broadly speaking, this only occurs once every five years. Thus, from the point of view of cultivation, controlling *Fusaria* is relatively unimportant in economic terms. The same argument applies to the further development of resistant strains. If relatively strict (i.e. low) mycotoxin concentration limits were to be imposed then this would render part of the current harvest unsaleable. This in turn would greatly enhance the economic significance of efforts to control *Fusarium.*

2.6.1 Chemical control

Fungicides that have been approved for use in the Netherlands for the control of *Fusaria* and other fungi contain the active substances tebuconazole, triadimenol and, more recently, strobilurin. The chemical control of *Fusaria* also poses certain difficulties, for a variety of reasons. These fungicides have little effect on *Fusaria*. The use of chemical agents to control *Fusaria* is only worthwhile when the crop remains damp for extended periods of time while it is in blossom. *Fusaria* can be controlled on the fragments of a previously harvested crop before sewing the wheat. Aside from this, fungicides are only effective (and then only to a limited extent) immediately before and after the cereal has blossomed.

While fungicides can reduce the pressure of infection on crop plants, it is doubtful whether large-scale use will reduce the production of toxins. The use of fungicides can induce stress in *Fusaria*, and *in-vitro* studies have shown that stress is one of the factors that stimulate toxin production (D'Me98, GZP00).

In addition, some fungicides can create a 'biological vacuum'. This is especially true of wide-spectrum fungicides like benzimidazoles, azoles such as tebuconazole and triadimenol, and strobilurins. It is then relatively easy for fungi that are less susceptible to the fungicide (which may include *Fusaria*) to colonise this 'biological vacuum'.

Actual examples of this phenomenon are known, but its exact role in the cultivation of cereals is unknown (Dek82). This may well have something to do with the current rise in the incidence of *Fusaria*.

2.6.2 Biological control

Studies carried out in the United States over the past 20 years have achieved little in terms of the biological control of *Fusaria*. In Europe, however, there have been several new developments in this field. There is considerable optimism with regard to progress over the long term.

2.6.3 Fusaria-resistant wheat

The use of crop improvement techniques has shown that it is possible to develop strains of wheat that are resistant to *Fusaria*. This characteristic is 'polygenic', which means that it is coded for by the joint action of several genes (Eeu95). This makes it difficult to cross different strains, in order to increase the yield. One partially resistant strain of wheat is available, in which *Fusaria* infestations are reduced by 60%.

2.6.4 Cultural measures

As a strategy for reducing the pressure from fungi, good agricultural practice is superior to purely chemical or biological control. This protection strategy has three primary goals. Firstly it aims to reduce the amount of infectious material by means of effective crop rotation, tillage and weed control. Secondly it focuses on reducing the risk of infection by selecting an appropriate strain of wheat, growing healthy crops, using the correct sewing density, leaving an optimum distance between rows, and using the correct amount of nitrogen fertiliser. Finally, there is an effort to control the infection by the appropriate use of fungicides, cleaning the grain and storing the grain under optimum conditions. Since it minimises the risk of diseases and plagues, good agricultural practice reduces the need to resort to chemical agents. In fields where maize has been harvested and there are plans to sew wheat in the coming year, it is essential that the stubble be well ploughed under (Bec97). A German study showed that the use of deep ploughing in arable fields containing maize stubble reduced *Fusaria* infestations in wheat. Mycotoxin concentrations in the subsequent wheat harvest were reduced by more than 90% (Bec97). Furthermore, when wheat was preceded by crops other than maize, its DON content was much lower than would otherwise have been the case (Bec97). The removal of infected plant fragments is, theoretically, an alternative to ploughing them under. However, it would appear to be very difficult to achieve in

practice. In addition, long-stalked strains are less susceptible to fungal infections than short-stalked strains, possibly because the ears are further away from the soil (GZP00).

2.7 Conventional farming and organic farming

A comparative study carried out in Germany revealed that samples of organically grown wheat contained considerably less DON than samples produced by conventional agriculture. The former contained 30-40 µg/kg and the latter 100-250 µg/kg (Bec97). It is unclear whether large-scale sampling would show the same degree of difference. One possible explanation of the differences is that organic farming does not include the cultivation of maize (see also 2.6.4). Another difference with conventional farming is that organically farmed wheat is sewn at a lower density. This allows the crops to dry more quickly, which reduces the growth of fungi.

2.8 Conclusion

Good agricultural practice can reduce the incidence of both *Fusarium* and DON. It is not possible for this approach to be implemented in full. In the next few years DON will therefore, to some extent, continue to be an unavoidable contaminant of wheat and, to a lesser extent, of other cereals.

Chapter 3

Toxicity

3.1 Introduction

Symptoms such as nausea, vomiting, abdominal pain, diarrhoea and headache have been described in individuals who have consumed cereals that were severely infested with *Fusaria* (Bha89, Bha97, Gro99, Li99, Ram89, Wan93). However, it is by no means certain that these symptoms were caused by DON. They may have been due to other *Fusarium* toxins, such as fuminosins. No systematic data is available on the effects of chronic, lower-level human exposure to DON. Effects have been seen in productive livestock, however (see section 2.5). In addition, the acute and chronic toxicity of DON has been studied in various species of experimental animals. Pigs appear to have the highest susceptibility to DON. Mice and rats are less susceptible, while chickens and ruminants are the least susceptible of all. Little is known regarding the kinetics of DON.

3.2 Acute toxicity

Oral LD_{50} values* in mice vary from 46 to 78 $mg/kg_{body\ weight}$ (Fio93, For87). The major acute symptoms are vomiting, diarrhoea and refusal of food. In addition, abnormalities have been reported in the kidneys, gastrointestinal tract, bone marrow and lymphoid tissues (Rot96). The DON loads involved in these cases were more than ten thousand times greater than those normally encountered by humans.

* This is the dose that is lethal to half of the individuals in a group of experimental animals.

The most striking effect of DON is vomiting, hence the name 'vomitotoxin'. Dogs and cats start vomiting at concentrations of 8 and 10 mg/kg_{feed} respectively (Hug99), and pigs at a slightly lower level of exposure, corresponding to feed with a DON concentration of 5 mg/kg_{feed}. These levels of exposure are also several thousand times greater than levels encountered in humans. A DON concentration in feed of 2 mg/kg had no observable effects on blood parameters, growth or organ functions (Eri98).

3.3 Subacute, subchronic and chronic toxicity

3.3.1 Effects on weight gain

Iverson and his co-workers exposed mice to DON for a period of two years (Ive95). The effect encountered at the lowest level of exposure was diminished weight gain. The feed used in this study had DON concentrations of 0, 1, 5 or 10 mg/kg_{feed}. Both males and females exhibited diminished weight gain at concentrations of 5 mg/kg_{feed} or 600 µg.$kg_{body\ weight}^{-1}$.day^{-1} and above. In the males, the highest dose that had no effect was clearly 1 mg/kg_{feed} or 110 µg.$kg_{body\ weight}^{-1}$.day^{-1}. The females' growth curve shows that, at 1 mg/kg_{feed}, both weight gain and food intake were not significantly lower (in statistical terms) than in the control group. Purely on this basis, 1 mg/kg_{feed} could be considered to be the highest dose that has no effect in females. However, the position of the latter curve between that of the control group and those of 5 and 10 mg/kg_{feed} shows a clear dose-response relationship. Here, the highest dose that had no effect appears to be less than 1 mg/kg_{feed} (see figure 3.1: figure 5 in Ive95). From the finding that DON diminishes weight gain without reducing feed intake it can be deduced that DON reduces food conversion.

In one 15-week study using mice, the feed contained amounts of DON that were equivalent to exposures of 0, 400, 800 or 1500 µg.$kg_{body\ weight}^{-1}$.day^{-1}. In the males, the highest dose caused a slight reduction in their food and water intake. None of the doses caused a significant reduction in weight gain. This did occur, however, in females exposed to 1500 µg.$kg_{body\ weight}^{-1}$.day^{-1} (Khe84).

In the course of four experiments, Tryphonas and his co-workers found no evidence of diminished weight gains in mice exposed to 250 µg.$kg_{body\ weight}^{-1}$.day^{-1} (Try86).

In rats, diminished weight gain occurred at DOM concentrations of 12 to 20 mg/kg_{feed}. This is equivalent to approx. 600 to 1000 µg.$kg_{body\ weight}^{-1}$.day^{-1} (Arn86). One 90-day study using rats found that an exposure of 20 mg/kg_{feed} (approx. 1000 µg.$kg_{body\ weight}^{-1}$.day^{-1}) produced a diminished weight gain (Mor85). The disruption of

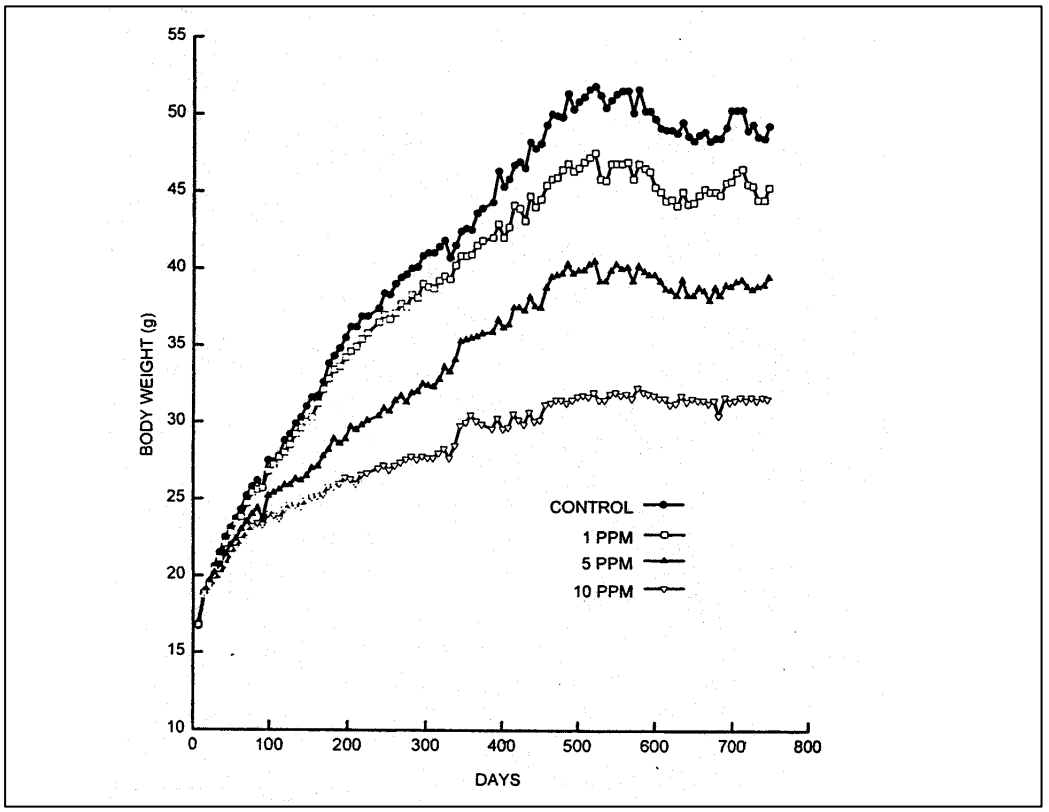

Figure 3.1 Body weight gains for female $B6C3F_1$ mice. (Source: Ive95)

nutrient uptake in the intestines by DON may account for the diminished weight gain in rodents (Hun91).

In separate 3-month study, piglets were given feed with a DON concentration of 0, 0.5, 1.0, 2.0 or 4.0 mg/kg_{feed}. This corresponds to intakes of 0, 20, 40, 80 and 160 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. At 2.0 mg/kg_{feed} or more, the rate of weight gain continued to decline during the first eight weeks. The rate reduction was dose-dependent. An exposure of 40 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ or less had no effect (Ber92). This is supported by the results of a second study (Ber93).

The effects described above occurred at continuous levels of exposure. The results of a study carried out by Banotai *et al*, using mice, show that the effects of DON are influenced by the exact exposure regime used (Ban99). They set up one group that was continually exposed to DON, and another group that was only exposed to DON on alternate weeks.* After only two weeks both groups exhibited diminished weight gain,

* The differences in effect between the 'continuous' group and the 'alternating' group are probably due to both a difference in the average exposure (20 ppm in the feed, as opposed to 10 ppm) in addition to the difference in the

relative to a control group. After four weeks, the average body weight in the 'alternating' group during DON-free periods was higher than in the group subjected to continuous exposure. When the alternating group received DON-free feed, the average weight in this group was higher than in the continuous exposure group. In the other weeks it declined to the same level as the latter group.

This study shows that, in the weeks when they were not exposed to DON, the alternating group made up the lost ground in terms of weight gains. Thus, the effect of DON on weight gain is, to some extent, reversible. The fact that the body processes and excretes DON within a few hours may account for this. The toxin is not accumulated in the body (Rot96).

3.3.2 *Effects on the immune system**

A third type of effect that has been observed at relatively low exposures to DON is an influence on the immune system. This can involve both suppression and stimulation. Suppression of the immune system can increase an individual's susceptibility to infectious diseases (Try86). In mice given feed containing DON, the proliferation of lymphocytes was found to be suppressed. Feed with a DON concentration of 2 mg/kg suppressed lymphocyte proliferation for five weeks. In animals given feed with a DON concentration of 5 mg/kg this effect persisted for one week (Rob88, Try86). This is supported by *in-vitro* studies (Mek01, Rot96).

A study carried out by Tryphonas *et al*, involved an experiment using mice that were exposed for 5 weeks to DON concentrations of 0, 250, 500 or 1000 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. In a test using *Listeria moncytogenes*, a pathogenic microorganism, there were mortalities in all of the groups. The four dosage groups experienced mortalities of 30%, 30%, 30% and 20% respectively. Mice in the control group survived for a little longer than those that had received DON. The study also reveals the effect of the bacterium on the levels of immunoglobulin in serum and on food intake. After four weeks, these effects were smaller in mice exposed to 500 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ than in those exposed to 1000 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (Try86). The researchers concluded that the highest level of exposure that had no immunotoxic effect was 250 to 500 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$.

Iverson and his co-workers exposed mice to DON for a period of two years (Ive95). In females, an exposure of 600 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ or more resulted in a

exposure regime (continuous versus alternating).

* The Committee has also examined the findings of Hara-Kudo *et al* (1996), as reported in the risk analysis completed for the *Nordic Council of Ministers* by Eriksen and Alexander (Eri98). The Committee has not taken this into account since only a summary is available, rather than a complete peer-reviewed report on the findings.

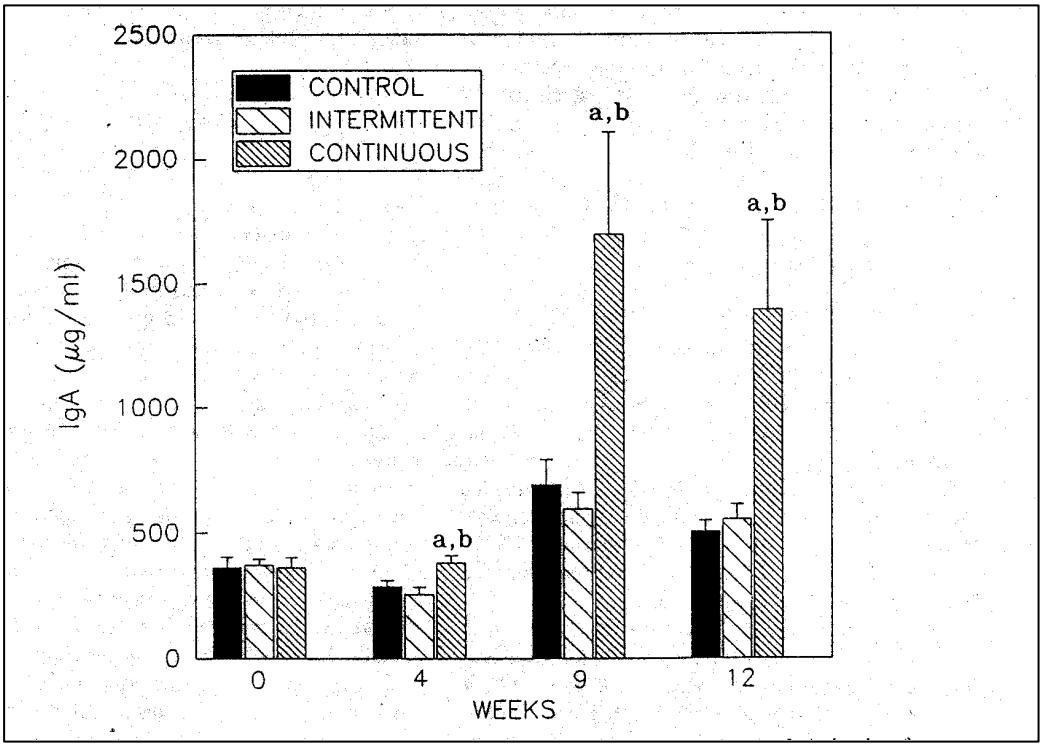

Figure 3.2 Effect of intermittent and continuous dietary vomitoxin exposure on serum IgA levels of $B6C3F_1$ mice compared with control. Data are means ± SE (eight to nine mice/group). [a] Indicates significantly different than control group at $P \leq 0.05$. [b] Indicates significantly different than other treatment group at $P \leq 0.05$. (Source: Ban99)

slight increase in the serum levels of immunoglobulins (Ig) A and G. In the males, there was a relative increase in liver weight*.

By affecting the transcription of DNA, DON is able to influence the serum levels of immunoglobulins (Zho99). In mice, the administration of feed with a DON concentration of 2 mg/kg had no observable effect on the serum levels of immunoglobulins (For86). A 24-week study using feed that contained higher levels of DON, namely 10 to 25 mg/kg, found a substantial elevation in the serum level of IgA, while there was a decrease in the levels of IgM and IgG. The reductions in immunoglobulin levels indicate suppression of the immune system. Cytokines may be involved in stimulating the production of IgA (Pes89, Rot96). Excessive production of IgA can contribute to the development of so-called immunological glomerulonephritis.

The effects described above occurred at continuous levels of exposure. The results of studies using alternating exposure are given below. These show that the effects of

* This is the weight of the liver as a percentage of total body weight.

DON are partly determined by the exposure regime itself. Female mice were continuously exposed to high levels of DON (20 mg/kg_{feed}) for a period of 13 weeks. The effects of this included an increase in the serum level of IgA to three times its normal value, deposition of IgA in the kidneys and symptoms of immunological glomerulonephritis (Ban99). The IgA levels in a group that was only given feed containing DON on alternate weeks were found to be little or no higher than those of the controls (see figure 3.2; figure 2 in Ban99).

3.3.3 *Effects on reproduction and development*

Reproductive disorders have been observed in various species of experimental animals following exposure to DON. Abnormalities of the foetal skeleton occurred when female mice were exposed to doses of 1000 to 5000 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ via a stomach tube. However, other studies using mice, rats, rabbits and pigs gave no indication of teratogenic effects (Eri98, Pie99).

The most significant reproductive effects reported were a slight reduction in fertility in female rats at an exposure of 2000 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (Mor85) and elevated foetal resorption in rabbits at 1800 and 2000 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (Khe86). One reproductive study using mice showed that exposure to 1500 to 2000 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ caused a loss of body weight and reduced food intake. There was also an increased level of infant mortality. Exposure to 380 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ produced no adverse effects (Khe84).

In cattle, DON did not appear to be secreted in the milk (Pre84, Pre87). Bottled baby formula based on cow's milk therefore contains no DON. Furthermore, in view of these findings, it is unlikely that human breast milk contains DON.

3.3.4 *Mutagenicity and carcinogenicity*

The results of a previously described chronic study (Ive95, see 3.3.1) give no indication that DON might be carcinogenic. Exposure of the skin to DON had no tumour-promoting effects (Lam95). Results are available from various *in vitro* mutagenicity tests. These include the Ames test, which uses various strains of *Salmonella*, *differential DNA repair* using K-12 strains of *E.coli*, and micronucleus tests using rat primary hepatocytes. None of these tests showed a positive response (Kna97, Weh78). Nevertheless, when Knasmüller *et al* applied the so-called chromosome aberration test to three *Fusarium* toxins that they were investigating (which included DON), they obtained a positive result (Kna97). In view of the relatively small number of negative results, the Committee considers DON to be non-mutagenic and non-carcinogenic.

3.4 Conclusion

DON has not been shown to have any effects on human subjects. In experimental animals, during the growth phase, the first effect to occur at low levels of exposure is a diminished weight gain. Effects on the immune system and on reproductive capacity only occur at higher levels of exposure. When alternating exposure is used, DON's effect on weight gain and on the immune system is smaller than in animals that are continuously exposed to the toxin. The Committee takes the view that DON has no effect on the development of cancer.

Chapter **4**

Tolerable daily intake (TDI)

4.1 Introduction

In the 1950s a need was identified for approval procedures for chemical substances such as food additives and chemical pesticides. At that time, with this need in mind, the concept of Acceptable Daily Intake (ADI) was developed. This is the highest lifelong exposure at which adverse effects on a given group of people can reasonably be excluded. One decade later the focus was on contaminants, particularly substances used by industry, such as PCBs, mercury and cadmium. The concept of Tolerable Daily Intake (TDI) was introduced in order to regulate the occurrence of these substances. The concept of the TDI is similar to that of the ADI.

4.2 NOAEL

When deriving a TDI, it is first necessary to find the highest level of exposure at which no observable effects have been recorded in studies (this is the No Observed Adverse Effect Level or NOAEL). The use of the word ‘observed’ indicates that only those effects that could be observed were taken into account; it is conceivable that certain effects will go unobserved. The word ‘adverse’ is added as a qualification, since not all effects are necessarily harmful in nature.* The preferred method of deriving a

* For example, if the metabolite of a substance is present in the urine then this will qualify as an effect, but it is not necessarily an ‘adverse’ effect. Another example of a non-adverse effect is the enlargement of the appendix in rodents following the intake of dietary fibre such as uncooked potato starch. This enlargement has been shown to be entirely

NOAEL is by means of a long-term study. 'Long-term' is a relative concept; for rodents this would correspond to a period of two years.

4.3 From NOAEL to TDI*

With many substances, little is known about how human sensitivity to the substance in question relates to the sensitivity of the experimental animal species that was used to derive a NOAEL for that substance. Thus, when attempting to establish a TDI, it is necessary to divide the NOAEL by what is referred to as an 'interspecies factor'. The value of this factor is always greater than one. This means that, to be on the safe side, humans are presumed to be more sensitive than the experimental animal to the substance in question. Furthermore, some people will be more sensitive than others. In order to protect the more sensitive individuals, once the NOAEL has been divided by the interspecies factor it is then divided by what is referred to as an 'intraspecies factor'.

When extrapolating from rodents to humans, an interspecies factor of 10 is generally used. A factor of two is generally applied to data derived from pigs, given the relatively high degree of physiological similarity between these animals and humans. An intraspecies factor of 10 is used. The TDI can therefore be derived by dividing the NOAEL obtained from mice (110 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ **) (see section 3.3.1) by 100. The value of the TDI will then be 1.1 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. The NOAEL in pigs – 40 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (see section 3.3.1) – is divided by 20. The study lasted only three months so, as is usual in these cases, an additional factor of two is used. This is a reflection of the uncertainty about whether short-term effects resemble long-term effects. Thus the value of the TDI derived from the study of pigs is 40 / (2 x 10 x 2) = $\mu g.kg_{body\ weight}^{-1}.day^{-1}$.

One underlying assumption of the above derivation of a TDI is that an exposure of x $mg.kg_{body\ weight}^{-1}.day^{-1}$ in a small animal will have the same effect as x $mg.kg_{body\ weight}^{-1}.day^{-1}$ in a larger animal or in humans. With some substances, however, the rate at which they are processed (which directly influences the effect) is not solely dependent on the exposure per kilogram of body weight, it is also influenced by metabolic rate. A measure of the latter is energy consumption per kilogram of body

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reversible, and should be seen as a physiological adaptation (Lee74).

* When no suitable data is available for deriving an NOAEL, an LOAEL (Lowest Observed Adverse Effect Level) can be used. This is the lowest level of exposure at which adverse effects are still observed. An additional uncertainty factor is then required in order to derive a TDI.

** As explained in chapter 3, the assumption that the NOAEL for female mice is less than 110 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ is quite defensible. Accordingly, the TDI that is based on this will be proportionately lower.

weight. This figure is higher in smaller animals than in larger animals. The body's total energy consumption does not increase in proportion to increases in total body weight. Roughly speaking, energy consumption is directly proportional to body weight to the power of 0.75 (GR85).

This can be discounted if a scaling factor is used. This reflects the relationship between energy consumption per kilogram of body weight in a given experimental animal, and the corresponding value in humans.* In addition, as with extrapolation on the basis of body weight, there may be other differences between species that also play a part in determining the effect of a given substance. That is why an interspecies factor is still required, in addition to the scaling factor.

Let us assume that a factor of 10 is generally used when extrapolating on the basis of body weight from rats or mice, for example, to humans. By contrast, a factor of three is sufficient for extrapolation on the basis of energy consumption for rats or mice - in addition to the scaling factor referred to above (GR85). Extrapolation on the basis of energy consumption actually removes part of the interspecies uncertainty. For this reason a lower interspecies factor can be used than the one required for extrapolation on the basis of body weight (GR85). Finally, as with extrapolation on the basis of body weight, an intraspecies factor of 10 is required.

The scaling factor in pigs is 0.9 (GR85). As described above, it is still necessary to use an interspecies scaling factor. In physiological terms, humans more closely resemble pigs than they do rats or mice. This is why an interspecies factor of just two is sufficient when dealing with data derived from pigs. Extrapolation of the data produced by Bergsjø required the use of an additional factor of 2, to compensate for the relatively short duration of the study (Ber93). Finally, a factor of 10 is used to allow for variation between individual people. When divided by 36 (= 0.9 x 2 x 2 x 10), the NOAEL derived from the study of pigs (40 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$) produces a TDI of 1.1 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. Data derived from mice is subjected to a scaling factor of 7, an interspecies factor of 3 and an intraspecies factor of 10. Thus the TDI based on the study using mice (Ive95) is 110 / (7 x 3 x 10) = 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$.

4.4 TDIs proposed by other bodies

TDIs have previously been derived for DON. In 1985, Kuiper-Goodman proposed TDIs of 3.0 and 1.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ for adults and children respectively (Kui85). These values are based on a NOAEL of 750 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (Khe82, Khe84). More recently, various bodies have proposed TDIs using extrapolation on the basis of body weight (Pie99, SCF99). The National Institute of Public Health and Environmental

* Accordingly, the respective scaling factors for mice and pigs are 7 and 0.9 (GR85). These are calculated as human body weight to the power of 0.75, divided by the body weight of the experimental animal in question to the power of 0.75.

Protection and the European Scientific Committee on Food have established TDIs for humans of 1.1 and 1.0 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ respectively (Pie99, SCF99). These values are primarily based on the study by Iverson *et al* (Ive95), and the resultant NOAEL of 110 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (Ive95).

4.5 Conclusion

Extrapolation on the basis of body weight results in a TDI of 1.1 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. This applies to data derived from both mice and pigs. According to extrapolation on the basis of energy consumption, studies of pigs and mice have produced TDIs of 1.1 and 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ respectively. One of the effects of exposure to DON concerns the rate of weight gain, and weight gain is related to the level of the metabolism. For this reason, the Committee takes the view that preference should be given to extrapolation on the basis of energy consumption. On further consideration, the Committee opted for the lowest TDI: 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. Chapter 7 explores the significance of this decision in terms of health.

Chapter 5

Exposure

5.1 Introduction

Effects in terms of weight gain are taken into account during the risk assessment of DON. Estimates of exposure should not take a short-term view, nor should they cover the entire life span. Instead, they should be based on intake during growth, particularly during those periods that are characterised by the highest growth rates. The exposure of young children thus becomes the central issue. Another reason for focusing on exposure in young children is that they have the highest food intake of any age group, per kilogram of body weight (GR01). Clearly, since cereal products are a major part of the diet of young children, this age group will also have the highest exposure to DON per kilogram of body weight.

Exposure estimates should ideally be based on measured concentrations in duplicated portions of people's actual diet. Such data is usually not available, however, and this is also the case with regard to DON. Nevertheless, there are estimates in which the concentrations in foodstuffs or in raw materials are combined with data on the consumption of these substances. This is the topic of this chapter. The calculations relate to the most important source of DON, namely wheat. The other cereals appear to contribute little towards the total DON intake (see table 5.1).

Table 5.1 DON concentration (µg/kg) in cereals and cereal-containing products, collected by the National Commodity Inspectorate in the period from September 1998 to January 2000.

	number of samples	median	av.±SD	%<detection limit[a]
food for babies, toddlers and young children	31	760	846±818	35
baking powder mixture	13	170	201±158	38
bread, rusks, crackers	18	194	195±70	11
barley	17	0	47±51	
oats	13	0	0±0	100
gingerbread, cake, pie	22	100	108±109	55
maize	6	195	308±374	17
muesli	13	0	82±127	69
pasta	9	210	194±132	22
rye	1	0	0	100
wheat	219	240	447±683	23
wheat flour	189	210	254±229	12
brans	26	180	288±469	27
starch, binding agents	5	0	48±67	80

5.2 RIVM (National Institute of Public Health and the Environment) reports about DON

RIVM issued a report about DON in 1999 and another in 2001. The first report covers the derivation of a TDI. The researchers also estimate the level of wheat consumption by combining data on the amounts of wheat contained in foodstuffs (Doo95) with estimates of the 1997/1998 Food Consumption Survey (FCS; Hul98).* If the TDI is divided by the 95th percentile of wheat consumption in children aged from one to four, this gives the DON percentage that, on average, wheat is permitted to contain (Pie99). This issue is examined in greater detail in Chapter 6.

The 2001 report contained estimates of exposure to DON (Pie01). On this occasion, instead of wheat concentrations in foodstuffs, the FCS data was combined with DON concentrations in foodstuffs, as measured by the National Commodity Inspectorate (see 5.4). The 2001 RIVM report also contained a so-called probabilistic estimate of the effect of the calculated exposure. The Committee acknowledges the

* The FCS describes food consumption, over a period of two days, by individuals of more than one year of age. The survey identifies 1211 different foodstuffs, in accordance with those set out in the Nevo Food Composition Table. Two hundred and fifty-eight of these products contain wheat (Pie99).

potential of this very promising method. However, it feels that the method has not yet been sufficiently widely used and evaluated to allow the results obtained by this means to be interpreted with adequate certainty.

5.3 DON concentrations in wheat and in foodstuffs

The concentrations of DON in wheat and wheat-containing products vary considerably. This is a result of differences in environmental conditions during cultivation of the wheat. The National Commodity Inspectorate has used HPLC (High Performance Liquid Chromatography) to measure DON concentrations in various types of foodstuffs and raw materials. Table 5.1 illustrates the data that was collected between September 1998 and January 2000. These figures were used by RIVM to calculate the exposure to DON (see 5.4 and table 5.2). Annex C also contains data gathered after January 2000.

These measurements demonstrate that the median DON concentrations of the various categories are lower than the arithmetically derived average concentrations. This means that the distribution of the DON concentrations for the various products is skewed. The same is true of many other dietary contaminants. In statistical terms, there are no significant differences between the concentrations in wheat and in flour, nor between the various wheat-containing foodstuffs. The median DON concentration in the group as a whole is approximately 200 µg/kg.

Conversely, statistical analysis shows that foodstuffs for babies, toddlers and young children contain significantly more DON than other products. The median concentration was approx. four times higher. One possible explanation for this difference is that different strains of wheat are cultivated for different purposes. Softer wheat is used for breakfast cereals, for example, while harder wheat is used for bread. In addition, the year in which the wheat was harvested also has an effect. With one exception, all of the samples for the category of foodstuffs for babies, toddlers and young children were collected in April and May 1999. Accordingly, these samples would have been produced using the wheat harvest of 1998. Table 6.2 and annex C show that the 1998 harvest contained considerably more DON than those of 1999 and 2000. It is therefore quite likely that young children were exposed to higher levels of DON in 1999 than was the case in previous or subsequent years. As mentioned above, the 1999 crisis of high DON concentrations in breakfast cereals resulted in an action limit of 500 µg/kg. Many companies have instigated preventative measures, and refuse to accept wheat lots with high DON concentrations. Some companies even refuse to accept lots with DON concentrations in excess of 100 µg/kg. For this reason, the Committee believes that the exposure level of young children to DON is now lower than it was in the past.

5.4 Exposure to DON

The data presented here by the Committee was obtained from RIVM estimates of exposure to DON (see also section 5.3; Pie01). This is based on the figures for DON concentrations in wheat-containing foodstuffs (given in table 5.1), classified into 14 groups. Not all of these foodstuffs were analysed to determine their DON content. Accordingly, those foodstuffs that were analysed were used as a basis for calculating the average DON content per group.* It was then assumed that the other products (the ones that had not been analysed by the National Commodity Inspectorate) in that group contained wheat with the same DON concentration (Doo95). Since this work is based on prepared foodstuffs, it takes account of possible changes in DON content resulting from storage and preparation.

The estimated DON concentrations were then combined with the FCS data, using the so-called 'STatistical Exposure Model' (STEM) (Slo93, Slo98) (table 5.2)**. Table 5.2 shows exposure estimates. These were obtained by using STEM to derive a value for exposure as a function of age.

Table 5.2 Average daily DON intake ($\mu g.kg_{body\ weight}^{-1}.day^{-1}$) by Dutch children in 1999, estimated on the basis of age using the STatistical Exposure Model (STEM) (Pie01).

age in years	1-2	2-3	3-4	4-5	6-8
median	1.4	1.3	1.2	1	0.8
P90	24	2.1	1.9	1.7	1.4

Table 5.2 shows that DON intake is lower in higher age groups. This data shows that exposure per kilogram of body weight is highest in 1-year-olds. For all age groups, the estimated median intake exceeds the TDI of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ that was derived in chapter 4.

A rough comparison of exposure to DON (table 5.2) and wheat consumption (table 6.1) shows that the average DON content of the foodstuffs consumed by those with a higher level of exposure is about the same as those with a moderate level of exposure. Thus a high level of exposure primarily results from a high level of wheat consumption. Accordingly, a reduction in the DON content of foodstuffs would not reduce the amount of exposure specifically for those with high exposure levels. Nevertheless, there would be a general reduction across the entire range of DON exposures.

* These calculations were based on the wheat content of the foodstuffs.

** STEM takes inter-individual variation in the consumption of foodstuffs into account. However, it makes no allowance for variation in the DON content of individual products, or of products within a given group.

Table 5.3 Contribution of groups of foodstuffs to DON exposure DON (%) (Pie01).

group	1-year-olds	2-4-year-olds
bread/rusks/crackers	53	64
food for babies/toddlers/young	22	6
gingerbread/cake/pie	9	11
pasta	3	4
mixed products	2	3
other wheat products	8	11
barley	0	0
maize	0	0
oats	0	0
rye	0	0
buckwheat	0	0

Cereals other than wheat make a negligibly small contribution to the average DON intake (table 5.3). In children aged one to two, 22% of the total exposure to DON is due to special foodstuffs for babies, toddlers and young children. Since the DON levels in these products were relatively high, a specific concentration limit could help to reduce these children's daily intake of the toxin. Twenty-two percent of a DON intake of 2.7 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ is equivalent to 0.6 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. If this group of foodstuffs, which is readily identifiable, had a DON content of 200 µg/kg product instead of 850 µg/kg product (table 5.1), then it is estimated that the DON intake would be 16% lower.

5.5 Conclusion

The Committee is aware that RIVM's estimates abound with uncertainties (annex D). It would be useful if these uncertainties could be reduced, or removed completely, as this would provide a firmer basis for formulating advice and policy with regard to DON. The Committee nevertheless considers that the data is sufficiently reliable for conclusions to be drawn regarding exposure to DON. However, the Committee points out that, as a result of measures that have been put into effect since 1999, current levels of exposure are probably lower.

Chapter 6

Concentration limits

6.1 Introduction

Some countries use concentration limits for DON in certain cereals. The American Food and Drug Administration uses a concentration limit of 1000 µg/kg for DON levels in wheat. Canada's limit is 2000 $\mu g/kg_{wheat}$, while Austria has a limit of 500 µg/kg for maize. The Netherlands has a so-called action limit for DON levels in cereal products. Until the end of 1999, this limit was 1000 $\mu g/kg_{wheat}$. Rather than being a statutory standard, this limit value has been agreed by industry and the government. Whenever the action limit is exceeded, an investigation is triggered to find out why this occurred. This does not necessarily mean that products with a DON content that exceeds the limit are automatically declared unfit for human consumption.

At the end of 1999, the Minister of Public Health, Welfare and Sports and the Minister of Agriculture, Nature and Fisheries acquainted themselves with the above-mentioned RIVM report on DON (Pie99). As a result of this, and in consultation with industry, the government provisionally reduced the action limit for DON in cereal products from 1000 to 500 $\mu g/kg_{wheat}$.

6.2 Concentration limits according to RIVM

Exposure to DON can be restricted by imposing limits on the amount of this toxin that foodstuffs are permitted to contain. How does one determine the limit that is required to prevent a given level of exposure from being exceeded? For this purpose, RIVM has

Table 6.1 Wheat consumption by children aged from one to four in the Netherlands in 1997/1998 (Pie99).

	boys (n=135)		girls (n=119)	
	g/day	$g.kg^{-1}.day^{-1}$	g/day	$g.kg^{-1}.day^{-1}$
average±SD	63±25	4.5±1.8	58±23	4.5±1.8
median	63	4.3	53	4
P95	101	7.3	103	8.5

'spread' the average of the acceptable exposure to DON in the critical period (in other words, the TDI) over the amount of wheat that young children consume (Pie99). In order to protect the majority of individuals in the critical group, it is necessary to take, for example, the 95th percentile as a basis, rather than the median wheat consumption in this group (Pie99; see also 5.2). Estimates based on the two days 'measured' in the FCS give the 95th percentile of daily wheat consumption in boys and girls aged from one to four as 7.3 and 8.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (table 6.1; Pie99) respectively.

The permitted average DON content of wheat is estimated by dividing the TDI by the estimated 95th percentile of normal wheat consumption. Thus 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (see 4.5) is divided by 8.5 g of wheat per kg of body weight*. Using this average content, at least 95% of the critical group will be 'protected'. The average content that is considered acceptable is therefore 60 $\mu g/kg_{wheat}$. RIVM has proposed that the maximum content to be considered acceptable for individual lots should be equivalent to this average permissible content (Pie99).

6.3 Concentration limits according to the Committee

With regard to the derivation of a concentration limit, the Committee considers that the approach described in 6.2 requires a number of modifications. Average exposure during the period of growth is fundamental to any risk evaluation of DON. For this reason it is also essential to find out the average intake of wheat over a protracted period of time, as well as the distribution of this intake between different individuals. RIVM used the average of the two-day FCS as an estimate of average wheat consumption over a protracted period of time (Pie99). However, the distribution of the individual two-day averages reflects not only differences between individuals in terms of habitual intake, but also differences between the two 'measured' days, per person. Consequently, the value of 8.5 $g.kg_{body\ weight}^{-1}.day^{-1}$ is an overestimate of the 95th percentile of the averaged individual levels of wheat consumption, over a protracted period of time.

* This relates to girls. This approach was adopted for purposes of safety, since this value is higher than for boys.

Table 6.2 Theoretical average DON contents (g/kg) in European wheat at different concentration limits (Source: Commodity Board for Cereals, Seeds and Legumes).

	1998 harvest, n=158		1999 harvest, n=586		2000 harvest, n=602	
	av.	%>limit	av.	%>limit	av.	%>limit
no limit	580	0	270	0	320	0
limit = 1250	450	15	250	1	260	2
limit = 1000	350	19	240	4	240	5
limit = 750	300	26	210	9	210	10
limit = 500	220	41	170	17	170	18
limit = 250	120	66	110	37	120	35
limit = 110[a]		84		67		59

[a] This is approximately the limit of detection.

Assuming that intra-individual variation is just as large as inter-individual variation, and using a previously described correction method (NRC86), the actual 95th percentile of intake over the longer term is estimated to be 16% less than the above-mentioned 8.5 $g.kg_{body\ weight}^{-1}.day^{-1}$.

In order to safeguard the average acceptable content, the content in individual foodstuffs is permitted to exceed this average. This is possible because some foodstuffs contain little or no DON. The Commodity Board for Cereals, Seeds and Legumes has data from the measurement of DON contents in lots of wheat supplied by milling companies. Table 6.2 shows that, when a given limit is adopted, the average content in the 'approved' lots is below the level of that limit. Using the table in question, the Committee estimates what limit is needed to achieve a given acceptable average content. This is based on the assumption that the distribution of DON contents, as described by the Commodity Board for Cereals, Seeds and Legumes (table 6.2), is representative of the situation in the Netherlands. Thus, if lots of wheat with a high DON content are mixed with low-content lots (thereby reducing the variation in DON contents), then there will be a smaller difference between the limit and the average DON content in the lots submitted for approval.

Table 6.2 shows that the DON contents in 1998 were much higher than in 1999 and 2000. The Committee is basing its work on estimates relating to all three years. However, a situation could arise in which a child consumed wheat from two bad harvests in the period between its first and fourth birthdays. Thus the Committee has elected to describe this situation, which is the most adverse of all.

In order to prevent an average exposure at the level of the TDI of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ in 95% of young children, wheat is permitted to have an average

DON content of only 70 µg/kg.* Table 6.2 can be used to derive the limit needed to achieve an average of 70 µg/kg. In all three years, a limit of 250 µg/kg would have produced an average content of about or somewhat more than 100 µg/kg, which is approximately the limit of detection. Thus, to achieve an average content of 70 µg/kg, the only lots that could be accepted would be those with a DON content either below or just above the limit of detection. Four fifths of all samples taken in 1998 and one third of those taken in 1999 and 2000 had a DON content that exceeded the limit.

The current action limit of 500 µg/kg means that 'acceptable' wheat has an average DON content of approximately 200 µg/kg (table 6.2). In that instance, it is estimated that the 95th percentile of DON exposure in young children would be approximately 1.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$.

If an exposure to DON of up to 2.5** $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ was considered acceptable for young children, then wheat could have an average DON content of 350 µg/kg.*** Table 6.2 shows that in a year such as 1998, a concentration limit of approximately 1000 µg/kg was sufficient for this purpose. In 1999 and 2000, there was actually no need to set a limit at all. This means that a concentration limit of as much as 1000 µg/kg (or even higher) for the DON content of wheat would prevent young children's exposure to this toxin from exceeding the level of 2.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. This would also apply to years in which the DON content of wheat equalled the levels seen in 1998.

If the requisite concentration limits are strictly applied to all wheat that is intended for human consumption, then it would not be necessary to bother with the DON contents of end products. In addition, there is always the option of setting concentration limits for the DON content of end products. The DON content of the wheat in wheat-containing products must not exceed the applicable concentration limits. Consequently, the concentration limit for DON in an end product can be determined by multiplying the above-mentioned concentration limits by the fraction of that product that consists of wheat. By way of illustration, if the permitted DON content of raw wheat is 300 µg/kg, then a product containing 33% wheat will be permitted to contain 100 µg/kg.

6.4 Conclusion

The current action limit for DON in wheat products, which has been agreed by government and industry, is 500 µg/kg. If applied to raw wheat, this limit should at least be sufficient to prevent exposures in excess of 2.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. The limit may

* Calculation: 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ / (0.84x8.5 $g.kg_{body\ weight}^{-1}.day^{-1}$) = 70 µg/kg.

** This level of exposure roughly corresponds to a) the 95th percentile of young childrens' exposure in 1999 and b) the TDI, if this were to be based on immunological effects (see 7.2.2).

*** Calculation: 2.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ / (0.84x8.5 $g.kg_{body\ weight}^{-1}.day^{-1}$) = 350 µg/kg.

also be of use in reaching decisions about whether or not to mix the wheat with other foodstuffs. A limit of approximately 100 μg/kg is required if exposures in excess of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ are to be avoided.

Chapter **7**

Concluding remarks

7.1 Introduction

The DON content of wheat is highly dependent on weather conditions during the growth and flowering of the cereal, as well as on the type of agricultural technique being used. There were adverse weather conditions in Western Europe during 1998. As a result, in 1999, the DON concentrations to which young children were exposed were higher than they had been for many years. In that year, half of all children aged from one to four were exposed to an average of 1.3 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ of the toxin. Moreover, 5% of them had an average DON exposure in excess of 2.4 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (see table 5.2).

In accordance with common practice, the Committee has derived a TDI for DON. The value obtained is 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ (see chapter 4). The above figures show that exposure in a consumption year such as 1999 was, in some cases, higher than the TDI. In theory, exposure can be reduced by 1) using cultivation measures to reduce the DON content of wheat 2) not using wheat with a high DON content for human consumption and 3) reducing wheat consumption.

It appears that the first approach can only be partly implemented, especially in the short term (see chapter 2 and table 6.2). With regard to the second measure, the Committee would like to draw attention to the action limit for DON in wheat products (500 µg/kg) that was agreed by government and industry in 1999. In addition, to be on the safe side, some wheat-processing foodstuff companies switched to even lower limits for their raw materials during 1999, or in subsequent years (see 5.3). The

Committee takes the view that a reduction in wheat consumption would only be indicated if the potential health benefits of doing so were to outweigh the advantages of maintaining present levels of consumption.

In this chapter, the Committee estimates what effects might be expected to appear if the TDI were to be exceeded by a given amount. In doing so, it has taken account of the character of the NOAEL/TDI model and the nature of the effects that have been observed in experimental animals. These points are reviewed in the light of wheat's importance in terms of public health.

7.2 Considerations

7.2.1 NOAEL/TDI model

At the time of its introduction, the model that is commonly used to derive a TDI from an NOAEL was intended to virtually eliminate the harmful effects of artificially introduced substances. Accordingly, as a consequence of the uncertainty factors used, the method used to derive a TDI can best be described as 'circumspect'. Thus an exposure that briefly exceeds the TDI on a few occasions will not necessarily produce adverse effects.

The Committee has, nonetheless, derived a TDI for DON. This is being used as the starting point for an examination of the acceptability of certain levels of exposure. The TDI was based on the NOAELs for diminished weight gains that were obtained from studies on mice (Ive95) and pigs (Ber94). Pigs were found to be more sensitive to DON than mice. A subchronic study in pigs found a NOAEL of 40 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$, while a chronic study in mice gave a value of 110 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. When extrapolated to allow for energy consumption, the pig study produced a TDI of 1.0 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$, while the mouse study gave a TDI of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$.* In accordance with common practice, the Committee adopted the lower value, just to be on the safe side. In physiological terms, pigs resemble humans more closely than do mice. Accordingly, the Committee considers that a TDI of 1.0 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$1 is truly justifiable. It shares this view with RIVM and the SCF (Scientific Committee on Food) (Pie99, SCF99).

7.2.2 Nature of the effects

No study has been carried out to determine whether DON actually diminishes weight gains in humans. All of the available data, however, suggest that Dutch schoolchildren

* The study in mice results in a lower TDI than the study in pigs, even though the NOAEL based the mice study is higher. This is explained by the larger scaling factor for mice, resulting from its much lower body weight.

are becoming ever taller and heavier (Fre00a, Fre00b). As has been pointed out, in 1999 five percent of young children had an exposure to DON that exceeded 2.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. Yet, even under these circumstances, no cases of diminished weight gain in young children were described. Since growth is influenced by a wide range of factors, however, the possibility that some children might have been affected cannot be excluded.

The Committee is unaware of any evidence of effects of DON on height, which is no less important than body weight as a measure of growth in humans. It is also important that the rate of growth is highest prior to birth, and continues to be relatively high during the first six months of life. This is reflected in the sensitivity to effects on growth and development. During the latter phase of life, an individual's diet includes little or no wheat. As a result, their exposure to DON is virtually zero.

Studies in mice have shown that alternating high exposure to DON produces more limited affects than does continuous high exposure to the toxin. This is particularly true of the effect on the immune system, and less so with regard to the effect on weight gain. This is important since people encounter alternating high exposure more often than continuous high exposure. This study has also shown that, during periods of little or no exposure, the body compensates for the effects of DON. A contributing factor here is that DON does not appear to accumulate in the body; it has a half-life of only a few hours.

While the Committee views the diminution of growth as an adverse effect, it does not regard a slight diminution of the growth rate as a cause for concern. On the basis of the contents of this section and of the preceding section, the Committee considers it unlikely that this effect will occur as a result of a few, short-lived exposures in excess of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$.*

The Committee considers effects on the immune system, on fertility and on the unborn child to be severe in nature. The NOAELs for these effects are 250 to 500 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. These are 2.5 to 5 times higher than those for diminished weight gain (see also chapters 3 and 4). As a result, a TDI based on effects on the immune system, fertility and the unborn child (as well as the usual uncertainty factors) will have a value of 2.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. This means that, up to this level of exposure, effects on the immune system, fertility and the unborn child can be reasonably excluded.

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* One of the factors that the Committee has taken into account here is that an exposure of 40 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ produced no inhibition of growth in the pig, the most sensitive experimental animal species.

7.2.3 *Importance of wheat for public health*

In accordance with the request for advice (Annex A), the Committee has also given due consideration to the importance of wheat in terms of public health. Wheat is a major component of foodstuffs such as bread, pasta and breakfast cereals. In the area of nutrition, it has been recommended that adults obtain at least 40% of their energy requirement in the form of carbohydrates (GR01). Since wheat is almost 80% starch (a complex carbohydrate), it makes an important contribution to the achievement of this recommendation. Bread provides 15% of young children's total carbohydrate intake, while other cereal products provide 10% (Hul98). One of the reasons behind the Health Council's previous recommendation that at least 40% of energy intake should be in the form of carbohydrates is that more energy consumed in the form of carbohydrates means less energy consumed in the form of fats. This in turn means that there may be less chance of individuals becoming overweight (GR01). The optimum form of nutrition for babies up to about six months of age is breast milk. Ideally, the diet of babies above six months of age should be supplemented with foodstuffs other than breast milk. The supplementary foodstuffs should preferably have a lower fat content than breast milk, so that at least 50% of the energy comes from carbohydrates. This means that wheat is also an excellent source of nutrients for children, starting at about six months of age.

Wheat is also an important source of protein. Bread provides 12% of the total protein intake, while other cereal products provide 6%. There is some evidence to suggest that vegetable protein, such as that found in wheat, is more beneficial to human health (in varied mixtures) than protein of animal origin (GR01). Furthermore, bread and other cereal products provide 25% and 16% respectively of total dietary fibre intake, 17% and 12% of total iron intake, 10% and 9% of total zinc intake, as well as 17% and 4% of total folic acid intake. Bread provides 40% of total iodine intake.

7.3 Conclusion and recommendation

The Committee concludes that there is virtually no risk of harmful effects from DON at exposures of up to 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. At exposures of between 0.5 and 2.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$, the risk of diminished weight gains is very small indeed, while effects on the immune system, fertility and the unborn child are still almost completely eliminated. Nevertheless, the Committee feels that exposure to toxins such as DON should be kept as low as possible. To this end, a guideline of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ is proposed. One possible approach is to reduce the DON content of wheat by the use of existing alternative cultivation measures, and the development of new ones. The series of measures proposed by the Commodity Board for Cereals, Seeds and Legumes

(GZP00) may provide a useful starting point in this regard. There are certain practical limitations to this approach, however. For instance, there must be international compliance with any measures of this kind. After all, the majority of wheat consumed in the Netherlands is imported from other Western European countries. The Committee also recommends that a system be developed to provide the earliest possible warning of elevated DON levels in wheat, such as those found in the 1998 harvest.

It is also important that the wheat-processing foodstuff companies treat wheat lots with elevated DON contents as unfit for human consumption. Nor should such wheat be mixed with lots that have lower DON contents. The decision to mix such lots, rather than to treat them as unfit for human consumption, represents a missed opportunity for reducing human exposure to DON. The current action limit for DON in wheat products, which has been agreed by government and industry, is 500 µg/kg. If applied to raw wheat, this limit should be sufficiently low to prevent exposures in excess of 1.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$. The limit may also be of use in reaching decisions about whether or not to mix the wheat with other foodstuffs. A limit of approximately 100 µg/kg would be required if exposures in excess of 0.5 $\mu g.kg_{body\ weight}^{-1}.day^{-1}$ are to be avoided. This is the same order of magnitude as the current limit of detection for DON.

Another way of reducing exposure to DON would be to reduce wheat consumption. Wheat products are very important in terms of public health, however. For this reason, the Committee advises against any reduction in wheat consumption.

The Hague, 23 October 2001,
for the Committee
(signed)

Dr LTJ Pijls,	Prof. JH Koeman,
Secretary	Chairman

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Annexes

Annex **A**

The request for advice

On 6 December 1999, the Minister of Public Health, Welfare and Sports, also on behalf of the State Secretary for Agriculture, Nature and Fisheries, asked the Health Council for its advice on DON. This request was as follows:

I hereby request, also on behalf of the State Secretary for Agriculture, Nature and Fisheries, that you produce an advisory document on the RIVM report entitled 'Deoxynivalenol; Derivation of concentration limits in wheat and wheat containing food products'.

Elevated concentrations of deoxynivalenol (DON) were found in one type of food last April. On several occasions since then, the Inspectorate for Health Protection, Commodities and Veterinary Public Health has found high concentrations of DON in several lots of cereals. DON is a mycotoxin produced by the fungus *Fusarium*, which occurs naturally on cereals. The acute effect of ingesting high concentrations of DON is vomiting. The most important effects in chronic exposure are reduced food intake and diminished growth, as well as effects on the immune system. For these reasons, RIVM has been asked to complete a toxicological background study within a short space of time. The results of this study will then be used as a foundation on which to base a new standard. The report on the RIVM report has now been received, and a copy is appended to this document.

On the basis of toxicological data, the RIVM report recommends a Tolerable Daily Intake (TDI) for DON of 1.1 µg/kg bw/day. On the basis of this TDI, the RIVM has subsequently calculated that the concentration limit for DON in wheat should be 120 µg/kg while the limit in bread should be 60 µg/kg. Observing such limits will ensure that the TDI is not exceeded. This is based on the principle of protecting the population group that is most at risk. In this case, that would be children aged from 1 to 4 who have

high levels of wheat consumption. The aim is that application of this standard will protect the 95th percentile of the population.

High concentrations of DON, in excess of the standards proposed by RIVM, have been found in various, recently sampled lots of cereals, flower and cereal-containing foodstuffs. There is considerable variation in DON concentrations, but it is by no means unusual to encounter concentrations that are 200% to 400% of the standard proposed by RIVM. The concentration limits proposed by RIVM are therefore exceeded in practice, and they are not feasible in the short term. RIVM points out that the limited effects of a minor breach of the proposed concentration limits for DON should be weighed against the importance of wheat in the range of foodstuffs that we consume. Given this consideration, the smallest possible temporary breach of these proposed concentration limits would be acceptable.

No conclusions have yet been reached regarding the long-term feasibility of specific concentration limits. The degree to which cereals are contaminated with the *Fusarium* fungus (and thereby with DON) is mainly dependent on weather conditions. In wet years, elevated concentrations of DON can be expected to occur. DON is very stable during the storage of cereals and when they are being processed into foodstuffs. As a result, a source-oriented approach is the only way to achieve reductions in the concentrations of DON in foodstuffs. Certain cultivation measures (such as deep ploughing, developing resistant strains and the like) can be used to reduce DON concentrations. In practice, however, it may not be possible to reduce the concentrations to the levels proposed by RIVM. In addition, the recommended concentration limit for DON coincides with current limits of detection for this toxin (60-100 µg/kg cereal or cereal product), which makes it difficult to reliably and reproducibly establish when the limits have been breached.

Given the complexity of this problem, I ask the Health Council to draw up a recommendation on the basis of the RIVM report. This should include a circumspect weighing-up of the advantages and possible drawbacks of the consumption of bread and cereal products. This consideration should take into account estimates of exposure, the potential harmfulness of DON at concentrations in excess of the proposed limit, the toxicological evidence in support of this, and the importance of bread and cereal products in the daily diet.

In December 1999, the toxicology of DON will be discussed by the European Commission's Scientific Committee for Human Nutrition. Please incorporate that committee’s conclusions into your own recommendation. In view of the fact that standards for DON will be imposed within the EU in the near future, I would ask that you treat this request as a matter of the greatest possible urgency.

Yours faithfully,

The Minister of Public Health, Welfare and Sports,
signed
Dr E. Borst-Eilers

Annex **B**

The Committee

- Prof. JH Koeman, *chairman*
  Emeritus Professor of Toxicology; Wageningen University
- Prof. VJ Feron
  Emeritus Professor of Toxicology; University of Utrecht
- Prof. J Fink-Gremmels
  Professor of Veterinary Pharmacy, Pharmacology and Toxicology; University of Utrecht
- Dr MRH Löwik
  Nutritionist/Epidemiologist; TNO Nutrition and Food Research Institute, Zeist
- Prof. GJ Mulder
  Professor of Toxicology; University of Leiden
- Prof. PJJ Sauer
  Professor of Paediatrics; University of Groningen
- Dr MA de Waard
  Phytopathologist; Wageningen University
- Dr JM de Stoppelaar, *advisor*
  Nutritionist/Toxicologist; Ministry of Public Health, Welfare and Sports, The Hague
- Dr RMC Theelen, *advisor*
  Ministry of Agriculture, Nature and Fisheries, The Hague
- Dr LTJ Pijls, *secretary*
  Nutritionist/Epidemiologist; Health Council, The Hague

Annex **C**

DON concentrations in wheat, wheat products and other cereals

In 1998, the National Commodity Inspectorate has begun measuring DON concentrations in a range of raw materials and foodstuffs. Some of the data that has been collected since then is shown in table 5.1. RIVM used the data in this table to estimate exposure to DON. The table below gives the DON concentrations that were found up to September of 2001. In addition to being specified in terms of year and product group, the data is also summed up over the years. The statistical significance of differences between years and between product groups are has not been estimated. The value of 50 has been entered for those DON concentrations that are below the limit of detection (approx. 100 µg/kg). The Committee points out that only a limited number of samples were taken in several categories during some years.

name	data	year of harvest				
		1997	1998	1999	2000	total
food for babies and toddlers	number of samples		28	16	5	49
	average		949	71	140	580
	standard deviation		796	31	81	737
	number < 100 µg/kg		8	13	1	22
	number > 500 µg/kg		17	0	0	17
self-raising flour	number of samples		35	31	110	176
	average		292	72	94	129
	standard deviation		440	53	87	223
	number < 100 µg/kg		8	25	75	108
	number > 500 µg/kg		3	0	1	4

name	data	year of harvest				
		1997	1998	1999	2000	total
bread, rusks, crackers	number of samples		12	17	22	51
	average		220	118	65	119
	standard deviation		77	126	35	103
	number < 100 µg/kg		0	9	18	27
	number > 500 µg/kg		0	1	0	1
pasta	number of samples		3	27	13	43
	average		187	65	124	91
	standard deviation		31	73	89	84
	number < 100 µg/kg		0	20	6	26
	number > 500 µg/kg		0	0	0	0
barley/pearl barley	number of samples		7	11	4	22
	average		50	45	50	48
	standard deviation		0	15	0	11
	number < 100 µg/kg		7	11	4	22
	number > 500 µg/kg		0	0	0	0
oats	number of samples		12	3	8	23
	average		50	33	50	48
	standard deviation		0	29	0	10
	number < 100 µg/kg		12	3	8	23
	number > 500 µg/kg		0	0	0	0
gingerbread, cake, pie	number of samples		19	44	14	77
	average		133	73	62	85
	standard deviation		96	66	32	75
	number < 100 µg/kg		7	35	12	54
	number > 500 µg/kg		0	0	0	0
maize	number of samples	2	4	1	1	8
	average	280	335	385	50	292
	standard deviation	28	471			326
	number < 100 µg/kg	0	1	0	1	2
	number > 500 µg/kg	0	1	0	0	1
muesli	number of samples		11	25	9	45
	average		89	72	91	80
	standard deviation		71	77	65	72
	number < 100 µg/kg		8	22	6	36
	number > 500 µg/kg		0	0	0	0
other cereals and products	number of samples				2	2
	average				50	50
	standard deviation				0	0
	number < 100 µg/kg				2	2
	number > 500 µg/kg				0	0
rice	number of samples				1	1
	average				50	50
	standard deviation					
	number < 100 µg/kg				1	1

name	data	year of harvest				
		1997	1998	1999	2000	total
rye	number of samples			8	2	10
	average			94	50	85
	standard deviation			69	0	64
	number < 100 µg/kg			6	2	8
	number > 500 µg/kg			0	0	0
wheat	number of samples	2	216	281	87	586
	average	50	467	161	168	274
	standard deviation	0	524	185	373	399
	number < 100 µg/kg	2	34	129	57	222
	number > 500 µg/kg	0	56	12	5	73
wheat flour	number of samples		22	17	44	83
	average		196	86	208	180
	standard deviation		90	58	250	194
	number < 100 µg/kg		2	11	18	31
	number > 500 µg/kg		0	0	5	5
brans	number of samples		9	8	3	20
	average		144	37	197	109
	standard deviation		107	23	145	106
	number < 100 µg/kg		4	8	1	13
	number > 500 µg/kg		0	0	0	0
starch	number of samples		9	9	2	20
	average		173	92	50	124
	standard deviation		116	54	0	96
	number < 100 µg/kg		3	5	2	10
	number > 500 µg/kg		0	0	0	0
total number of samples		4	387	498	327	1.216.
total average		165	397	124	126	212
total standard deviation		134	512	152	225	350
total number < 100 µg/kg		2	94	297	214	607
total number > 500 µg/kg		0	77	13	11	101

Annex **D**

Uncertainties in estimates of DON exposure

It is important to review the extent to which the National Commodity Inspectorate data is truly representative. In certain situations, this service takes on an investigative function. It issued instructions to those sampling wheat-containing foodstuffs for DON analyses that rather than seeking out 'suspect' lots, they should sample at random. Because this procedure lacked a sampling protocol, however, the values obtained by the National Commodity Inspectorate may have overestimated or underestimated the actual concentrations of this toxin in the Netherlands.

Representativeness is partly determined by the number of samples analysed. In the case of wheat and wheat flour, this number was large enough (table 5.2). The Committee takes the view that, in the case of the important group of bread/rusks/crackers (see table 5.4), the number of samples taken (n=18) was too limited to provide a representative view of this relatively heterogeneous group of foodstuffs in the Netherlands. The data in question may well overestimate or underestimate the actual situation.

Several uncertainties are linked to the use of the FCS, such as its use in estimating exposure to DON. These include the use of two independent sources of data rather than direct measurements of duplicate foodstuffs. There is also the extrapolation of two-day consumption data to indicate usual intake. Other doubts are associated with the under- or over-reporting of consumption, individuals reporting their own body weight, and the lack of data concerning children under one year of age. Some uncertainties more specifically concern estimates related to DON. These are the variation between

the results of chemical analyses, values that lie below the limit of detection and the use of a conversion table that has not been specifically validated for wheat.

Whereas the National Commodity Inspectorate bases its reports (see 5.3) on the HPLC method, the Commodity Board for Cereals, Seeds and Legumes uses ELISA (see chapter 6). Ring studies have shown that there is considerable variation between the estimates of DON content produced by different methods of analysis. However, there is no clear evidence that HPLC and ELISA systematically produce widely varying results.

DON concentrations of less than 100 µg/kg are difficult to verify, either qualitatively or quantitatively. In the data that it supplied to RIVM, the National Commodity Inspectorate viewed all concentrations of less than 100 µg/kg as equal to zero. While this implies that there was some underestimation of exposure, it would not have been very significant. This is because foodstuffs with these concentrations of toxin make only a limited contribution to the total exposure to DON.

With regard to the above-mentioned uncertainties, without additional information it is impossible to say with any certainty in which direction and to what extent these can be elaborated. In order to clarify this issue, the Committee recommends that the recent RIVM study (Pie01) be extended to include estimates of exposure based on other assumptions than those that are in use at the moment. These sensitivity analyses provide insight into a range of issues, including the robustness of the present estimates.