RIVM report 601516012/2004

Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment
Part IV

Editors: C.E. Smit and M.T.M. van Raaij

Contact: C.E. Smit
Expert Centre for Substances
ce.smit@rivm.nl

This investigation has been performed by order and for the account of the Board of Directors of the National Institute for Public Health and the Environment, within the framework of project 601516, Kennislacunes risicobeoordeling.

Authors

Chapter 1: M.E.J. Pronk
Chapter 2: S.M.J.G. Pelgrom

Abstract

Factsheets for the (eco)toxicological risk assessment strategy of the National Institute for Public Health and the Environment - Part IV

This report contains two factsheets describing risk assessment methods used at the Centre for Substances and Integral Risk Assessment (SIR) of the National Institute for Public Health and the Environment (RIVM). The main aim is to enhance transparency and consistency in the risk assessment methods used at SIR-RIVM. The factsheets on forestomach tumours and Leydigcell tumours describe the mechanistic aspects involved in tumour formation, and the similarities and differences in anatomy, physiology and exposure conditions between rodents and humans. An approach is presented to evaluate the relevance of these types of tumours for human risk assessment. The two factsheets reflect a state-of-the-art approach and are meant to facilitate discussion with other national and international parties involved in risk assessment.

Keywords: human risk assessment; forestomach tumours; Leydigcell tumours

Rapport in het kort

Factsheets voor de (eco)toxicologische risicobeoordelingsstrategie van het Rijksinstituut voor Volksgezondheid en Milieu - Deel IV

Dit rapport bundelt twee factsheets waarin methodieken worden beschreven die worden gebruikt voor de risicobeoordeling van stoffen bij het Centrum voor Stoffen en Integrale Risicobeoordeling (SIR) van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM). Het voornaamste doel is om de inzichtelijkheid en eenduidigheid van de bij RIVM-SIR gevolgde methodieken te vergroten. De factsheets over voormaagtumoren en Leydigcel tumoren beschrijven de mechanistische aspecten van de tumorvorming en gaan in op de verschillen in anatomie, fysiologie en blootstelling tussen knaagdieren en mensen. Er wordt een raamwerk geboden voor het evalueren van de relevantie van deze tumoren voor de humane risicobeoordeling. De factsheets vormen de weerslag van de huidige stand van wetenschap. Ze zijn bedoeld om de discussie met andere (inter)nationale partijen op het gebied van risicobeoordeling te bevorderen.

Trefwoorden: humane risicobeoordeling; voormaagtumoren; Leydigcel tumoren

Preface

This report was written within the framework of the project 'Kennislacunes Risicobeoordeling' (*'Knowledge gaps in risk assessment'*). The factsheets presented in this report have been reviewed by members of the peer review groups of the Centre for Substances and Integral Risk Assessment (SIR), and in some cases experts were consulted. The following persons are acknowledged for their contribution: M.E. van Apeldoorn, R. Beems, J. van Benthem, S. de Boer, A.G.A.C. Knaap, F.X.R. van Leeuwen, M.T.M. van Raaij, and P. Wester.

Contents

Samenvatting **6**
Summary **7**
Introduction **8**
1. Forestomach tumours **9**
2. Leydig cell tumours **23**

Samenvatting

In dit rapport worden twee factsheets gepresenteerd die worden gebruikt voor de beoordeling van stoffen bij het Centrum voor Stoffen en Integrale risicobeoordeling (SIR) van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

In het voormaagepitheel van ratten, muizen en andere knaagdieren worden na blootstelling aan chemische stoffen regelmatig tumoren aangetroffen. De factsheet '**Voormaagtumoren**' biedt een raamwerk voor het vaststellen van de relevantie van deze tumoren bij knaagdieren voor de humane risicobeoordeling, gegeven het feit dat de mens niet beschikt over een voormaag en het specifieke voormaag-epitheel ('squamous epithelium') niet in de menselijke maag voorkomt. Dit type epitheel komt echter voor in het bovenste deel van het menselijke spijsverteringskanaal, met name in de slokdarm. De factsheet beschrijft de mechanistische aspecten van de vorming van voormaagtumoren en de overeenkomsten en verschillen in anatomie, fysiologie en blootstelling tussen knaagdieren en mensen. Er wordt een getrapte benadering voorgesteld, aan de hand van het al dan niet genotoxisch zijn van een stof (eerste stap) en de toedieningsroute via welke voormaagtumoren veroorzaakt worden (stap 2). Op deze manier wordt een handreiking gedaan om te beoordelen of een stof, die bij knaagdieren voormaagtumoren veroorzaakt, ook voor de mens als potentieel carcinogeen moet worden beschouwd.

De factsheet '**Leydig cel tumoren**' geeft een raamwerk voor het vaststellen van de relevantie van het vóórkomen van deze tumoren bij dieren voor de humane risicobeoordeling. Het mechanisme achter de vorming van dit type tumoren wordt beschreven. Daarnaast wordt ingegaan op de overeenkomsten en verschillen tussen dier en mens voor wat betreft de anatomie en regulering van het hypofyse-hypothalamus-testikel hormoonsysteem. De factsheet definieert de omstandigheden waaronder Leydigcel tumoren kunnen worden beschouwd als niet-relevant voor de humane risicobeoordeling.

Summary

This reports presents two factsheets for the risk assessment methods used in the Centre for Substances and Integral Risk Assessment (SIR) of the National Institute for Public Health and the Environment (RIVM).

In the forestomach squamous epithelium of rats, mice and other rodent species tumours are observed frequently after exposure to chemicals. The factsheet '**Forestomach tumours**' provides a strategy for establishing the relevance of these tumours in rodents for human risk assessment, given that humans do not have a forestomach nor squamous epithelium in their stomach. Squamous epithelium is, however, found in the upper digestive tract of humans, in particular the oesophagus. The factsheet describes the mechanistic aspects involved in forestomach tumour formation, as well as the similarities and differences in anatomy/physiology and exposure conditions between rodents and humans. A tiered approach is presented: in the first tier, a distinction is made between genotoxic and non-genotoxic substances, followed in the second tier by a distinction between routes of administration by which a substance induces forestomach tumours. Guidance is thereby provided on whether a rodent forestomach carcinogen is considered to present a carcinogenic hazard to humans or not.

The factsheet '**Leydig cell tumours**' provides a strategy for establishing the relevance of these tumours in animal studies for human risk assessment. In this factsheet the mechanistic actions involved in the formation of Leydig cell tumours are described as well as similarities and differences in anatomy and regulation of the hypothalamic-pituitary-testicular axis (HPT-axis) between animal species and humans. In the strategy, it is defined under which conditions Leydig cell tumours in animal studies can be considered as not relevant for human risk assessment.

Introduction

One of the main tasks of the Expert Centre for Substances (SEC) and the Centre of Substances and Risk Assessment (SIR) of the National Institute for Public Health and the Environment (RIVM) is to assess the risk of compounds for public health and the environment. The availability of adequate and up-to-date risk assessment methods is of the highest importance to fulfill this task. Some of these methods follow international guidance, but many have been developed within the RIVM during the process of evaluation. These risk assessment methods are not rigid procedures but can be adapted based on new/developing scientific information, possibly triggered by questions from policy makers or by developments in (inter)national organisations.

For specific problems or gaps in the assessment of (eco)toxicological effects, 'factsheets' are written by employees of SEC and SIR in co-operation with experts. These factsheets describe the current assessment strategies of SEC and SIR, and their main aim is to provide a transparent and accessible guidance for issues that are not covered by regular guidance documents. After adoption of the factsheet by the advisory board and the head of the laboratories SEC or SIR all employees of SEC and SIR have to follow the risk assessment method described in the factsheet.

In 2001, the first eight factsheets were published in an RIVM report[1], followed by similar reports in 2002 and 2003[2,3]. The present report contains two factsheets that were produced in 2003 by SIR:

1. Forestomach tumours
2. Leydig cell tumour

We hope that by publishing these factsheets, the risk assessment methods followed by RIVM/SEC and /SIR will become more transparent. The authors of each factsheet have tried to describe the state of the art of their subject.
Remarks, omissions or supplementary information will be appreciated and can be send to ce.smit@rivm.nl and will be passed on to the responsible authors.

[1] Luttik R, Van Raaij MTM, editors. Factsheets for the (eco)toxicological risk assessment strategy of the Netional Institute for Public Health and the Environment (RIVM). Bilthoven: National Institute for Public Health and the Environment; 2001. Report no. 601516007.
[2] Luttik R, Pelgrom SMJG, editors. Factsheets for the (eco)toxicological risk assessment strategy of the Netional Institute for Public Health and the Environment. Part II. Bilthoven: National Institute for Public Health and the Environment; 2002. Report no. 601516009.
[3] Luttik R, Van Raaij MTM, editors. Factsheets for the (eco)toxicological risk assessment strategy of the Netional Institute for Public Health and the Environment. Part III. Bilthoven: National Institute for Public Health and the Environment; 2003. Report no. 601516010.

1. Forestomach tumours

Factsheet FSV-011/00, date 25-09-2003

Author: M.E.J. Pronk

Contents

1.1 Introduction and problem definition 10
1.2 Background information on forestomach anatomy, tumour types and characteristics, and normal incidences 10
1.2.1 Anatomy 10
1.2.2 Tumour types and characteristics 11
1.2.3 Normal incidences 11
1.3 Mechanism of forestomach carcinogenesis 12
1.3.1 Morphogenesis 12
1.3.2 Mechanistic considerations 12
1.4 Sensitive species/groups 14
1.4.1 Interspecies and sex differences 14
1.4.2 Intraspecies differences 15
1.5 Miscellaneous / Other considerations 15
1.5.1 Similarities and differences of oesophagus and oesophageal region of the stomach between humans and rodents 15
1.5.2 Lack of effect in the oesophagus 16
1.5.3 Markers/Threshold 16
1.6 Assessment and RIVM/SIR strategy 17
1.6.1 Approach taken by other organisations 17
1.6.2 RIVM/SIR strategy 18
References 20

1.1 Introduction and problem definition

The forestomach squamous epithelium of rats and mice and other rodent species is a common target site for tumour formation by chemicals. Forestomach epithelial tumours are most frequently induced when the chemical is given by the oral route, especially by gavage. Some chemicals, though, exert their carcinogenic potential also when administered by inhalation or by the parenteral route. Among the forestomach carcinogens identified are *N*-nitrosocompounds, polycyclic aromatic hydrocarbons, halogenated hydrocarbons, phenolic compounds. Some forestomach carcinogens have marked irritant properties. Many forestomach carcinogens act primarily through genotoxic (DNA-reactive) mechanisms, often also inducing tumours in other organs and tissues. For some others, however, evidence exists that the mode of carcinogenic action may include some non-genotoxic mechanism(s) [11,12,13,36].
Since humans do not have a forestomach nor squamous epithelium in their stomach, the relevance of neoplasia of the rodent forestomach for human risk assessment can be questioned. On the other hand, the forestomach squamous epithelium in rodents is a continuation of the squamous epithelium in the oesophagus. As the epithelium of the human oesophagus is morphologically of the same type as the oesophagus and forestomach epithelium of rodents, carcinogens targeting the forestomach squamous epithelium in rodents might therefore induce tumours in the human oesophagus (and related organs as oral cavity and pharynx). This factsheet provides a strategy for establishing the relevance of rodent forestomach tumours for human risk assessment.

1.2 Background information on forestomach anatomy, tumour types and characteristics, and normal incidences

1.2.1 Anatomy

The rodent stomach consists of two anatomically distinct parts of approximately equal size: a non-glandular forestomach and a glandular stomach. The forestomach is connected to the oesophagus at the gastro-oesophageal junction, and is clearly separated from the glandular stomach by a distinct border called the limiting ridge. This limiting ridge is a sphincter-like constriction that regulates movement of ingested material between the different sections of the stomach. The forestomach is not present in e.g. humans, monkeys, dogs, cats, rabbits and guinea pigs, but in pigs the pars oesophagea is of a similar structure. The main function of the forestomach is storage and triturition of ingested food prior to digestion in the glandular stomach. Under normal circumstances, reflux of gastric contents into the oesophageal lumen is prevented by the gastro-oesophageal junction that serves as a physiological sphincter.
The rodent glandular stomach comprises fundic and pyloric regions and is structurally and functionally similar to the stomach of other mammalian non-ruminant species, including humans [1,2,3,4].

Histologically, the oesophagus and stomach have the same general structure consisting of a mucous membrane (mucosa), a layer of fibrous connective tissue (submucosa), circular and longitudinal layers of striated or smooth muscle (muscularis externa) and a thin layer of fibrous tissue covered by mesothelium (serosa).
The mucosa (subdivided into an epithelial layer lining the luminal surface, the lamina propria and the muscularis mucosae) varies between the oesophagus and forestomach on the one hand

and the glandular stomach on the other, while the other layers are similar albeit variable in thickness. The main difference is that both the oesophagus and forestomach are lined with stratified squamous epithelium of which the most luminal layer of cells are keratinised, while the glandular stomach epithelium is columnar and contains many mucosal glands that secrete mucus, digestive enzymes and hydrochloric acid. Unlike the oesophagus of most mammals, including humans, the rodent oesophagus does not contain mucosal glands that secrete mucus (and some enzymes) to facilitate the passage of food. The forestomach also does not contain mucosal glands.
The limiting ridge in the stomach of rodents is a raised fold of forestomach mucosa. It forms an elevated border as a result of folding at the junction between squamous and columnar epithelium. In other mammalian species an abrupt change from stratified squamous epithelium to simple columnar is seen at the gastro-oesophageal junction [2,4,5].

1.2.2 Tumour types and characteristics

Forestomach tumours can be divided in epithelial and non-epithelial tumours. Attention is focussed only on the epithelial tumours, since the squamous epithelium is the main target of forestomach carcinogens.
There are two main classes of epithelial forestomach tumours: squamous cell papillomas (benign) and squamous cell carcinomas (malignant). Chemical-induced tumorigenesis of the forestomach squamous epithelium generally appears to be a continuum, progressing from hyperplastic lesions to benign and eventually malignant neoplasia. It may be difficult to differentiate between the various stages.
Squamous cell papillomas are usually exophytic, wart-like growths composed of a branching fibrovascular core covered by well-differentiated, hyperplastic squamous epithelium. Typically, they are supported by a stromal stalk continuous with the lamina propria, but some papillomas are sessile. Hyperkeratosis and acanthosis often occur (sometimes also parakeratosis and dyskeratosis), but some papillomas primarily show proliferation of the basal cell layer. They do not invade the gastric wall and do not metastasise. When multiple papillomas are diffusely spread throughout the forestomach, the term papillomatosis is sometimes used.
Squamous cell carcinomas generally have an exophytic growth pattern and are predominantly sessile. They are composed of elements of squamous epithelium with moderate or marked atypia and pleomorphism and, very important, also show invasion of tumorous squamous cells through the muscularis mucosae into deeper layers. There might also be invasion to adjacent tissues/organs and/or metastases to distant tissues/organs. Inflammation, ulceration and necrosis usually occur in carcinomas. Endophytic carcinomas are occasionally observed with penetration of the muscle layer and ulceration to various depths. Based on the degree of differentiation, squamous cell carcinomas can be characterised as well differentiated (with a tendency for differentiation like in normal squamous epithelium, varying degrees of keratinisation, and the presence of keratin pearls) or poorly differentiated (cellular and nuclear atypia, solid sheets of cells separated by thin stromal tissue or strands or groups of cells intermingled with connective tissue, minimal or no keratinisation) [1,2,3,6,7].

1.2.3 Normal incidences

Spontaneous squamous cell papillomas and carcinomas in the forestomach of rats and mice are rare findings, even in old animals. In rats, forestomach squamous cell papillomas have a very low incidence, usually less than 1%. Forestomach squamous cell carcinomas occur at a similar incidence. In mice, similar incidences have been noted for the malignant carcinomas, while the incidences for the benign papillomas were slightly higher (up to 4%). In both rats and mice, no

sex difference in incidence has been found for either the benign or malignant tumours [6,8,9,10,37].
In hamsters the occurrence of squamous cell carcinomas is rare, whereas the occurrence of squamous cell papillomas is much higher (incidences varying from 1 to 12%) than in rats and mice, but again without apparent sex differences.
Epithelial hyperplasia of the forestomach also occurs spontaneously in hamsters, which seem to be extremely sensitive to dietary compositions [6].

1.3 Mechanism of forestomach carcinogenesis

1.3.1 Morphogenesis

In general, the sequential development of forestomach tumours is morphologically characterised by early lesions such as slight focal epithelial damage with an inflammatory response, increased mitotic activity and diffuse hyperplasia with acanthosis and hyperkeratosis. Sometimes the hyperplasia is more complex with various degrees of basal cell proliferation. The early lesions progress to severe diffuse hyperplasia (with acanthosis, hyperkeratosis and sometimes parakeratosis), dysplasia, papilloma and/or papillomatosis and squamous cell carcinoma. Especially hyperplasias and papillomas containing a significant proliferation of basal cells and/or with evidence of atypia may be more prone to progress to squamous cell carcinoma.
Tumours may arise in any part of the forestomach, but a common location is near the limiting ridge. The time between the onset of treatment and the appearance of tumours varies considerably (from a few months to over one year), depending on the chemical, the dose level and duration of treatment [1,2,11,14].

1.3.2 Mechanistic considerations

The precise underlying mechanism of action for any forestomach carcinogen is at present not fully known. The tumorigenic lesions in the forestomach may be the result of a direct, genotoxic action of the compound on the epithelium, an indirect action (a prolonged proliferation stimulus) or a combination of both. Whatever the mechanism(s) involved, the tumours that arise in the forestomach epithelium are histologically indistinguishable.

1.3.2.1 Genotoxicity / non-genotoxicity / combination

Many forestomach carcinogens appear to act through a direct mode of action by inducing genetic alterations. They interact with forestomach DNA by alkylating DNA bases or forming DNA adducts. Their ability to influence DNA sequences such as those in oncogenes leads to populations of cancer precursor cells. Almost all DNA-reactive (or genotoxic) agents are multi-site carcinogens, also inducing tumours at sites other than the forestomach [14,36]. There is, however, also a number of forestomach carcinogens that do not interact with DNA and thus lack genotoxicity. This group includes the widely investigated compounds butylated hydroxyanisole (BHA), propionic acid and chlorothalonil. In general, non-genotoxic carcinogens lack initiating activity but possess (strong) promoting activity in their target organs. They appear to cause forestomach tumours primarily through initial cytotoxicity (as evidenced by necrosis, inflammation, erosion and ulceration) and subsequent sustained cell proliferation and hyperplasia. Generally it requires high dose levels and a long time for the development of carcinomas. The potential to induce necrosis, erosion, and ulceration differs between chemicals, and a positive association appears to exist between cytotoxicity and carcinogenicity [13,18,36].

Studies on promotion, inhibition and co-carcinogenesis have revealed that irritation, ulceration and hyperplasia of the forestomach squamous epithelium promoted the tumorigenic effect of classical genotoxic forestomach carcinogens like *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Cell proliferation may thus make an important contribution to forestomach carcinogens acting through a genotoxic mechanism. In turn, continuous strong cell proliferation makes the forestomach epithelial cells particularly susceptible to initiating agents [11,13,36].

1.3.2.2 Cytotoxicity and sustained cell proliferation

The importance of cytotoxicity and continued strong cell proliferation in forestomach carcinogenesis can be linked to the big regenerative potency of the forestomach mucosa, being a tissue that is exposed for long periods to acid and potentially abrasive food constituents. Abnormal continuous stimulation of these regenerative processes by mechanical and/or chemical irritation can result in extensive hyperplasia and tumour formation [27]. For example, for chemicals inducing forestomach tumours after gavage administration it was demonstrated that there is a correlation between early forestomach mucosal cell proliferation and hyperkeratosis and the development of neoplasia [15].
In general, cellular proliferation secondary to injury is associated with enhanced susceptibility to chemical carcinogenesis at the site of cell proliferation, because cells in an abnormally high proliferative state are rendered more vulnerable either to initiating agents present in ingested material or formed endogenously or to defects in cell reproduction, thus providing the stimulus for progression to neoplasia [11,14,16]. Studies with non-genotoxic forestomach carcinogens have demonstrated that persistent tissue damage and sustained cell proliferation are causally related to tumour development at this site. Tissue damage may be direct, as with chemical or mechanical irritants, or indirect, where damage to the forestomach cells may result from a breakdown of the gastric mucosal barrier [14,16,27]. During continuous strong cell proliferation caused by non-genotoxic compounds (e.g. phenolic compounds like BHA), forestomach epithelial cells are particularly susceptible to carcinogens: in that situation small amounts of genotoxic compounds (such as hydroquinone metabolites, quinone metabolites, active oxygen species or food-derived mutagens) may interact with forestomach DNA and result in forestomach cell transformation [17,18].

1.3.2.3 Reversibility of forestomach lesions

In general, forestomach lesions induced by genotoxic carcinogens do not regress but rather develop into papillomas and squamous cell carcinomas after cessation of treatment [17]. For some genotoxic forestomach carcinogens (e.g. methyl bromide), however, irritation rather than genotoxicity is largely responsible for the induction of hyperplastic lesions. In these cases hyperplastic lesions tend to regress after discontinuation of treatment [12,20].
Depending on dose and exposure duration, most forestomach hyperplasias as well as papillomas induced by non-genotoxic carcinogens rapidly regress after cessation of chemical treatment. Indeed, for phenolic compounds (which usually possess very weak initiating but very strong promoting activity) simple or papillary hyperplasia clearly regressed after treatment stopped. However, with some phenolic compounds (caffeic acid, sesamol, 4-methoxyphenol, but not BHA) atypical hyperplasia with a high level of DNA synthesis was observed, even after their withdrawal. Hence, even with non-genotoxic forestomach carcinogens it is possible that during strong proliferation genetic changes in DNA occur (e.g. due to interaction of the various compounds in the diet, which may result in the formation of DNA-reactive compounds in the forestomach), thereby causing the transformed cells to develop into atypical hyperplasias and finally carcinomas [17,18].

1.3.2.4 Oral administration versus other routes of administration

Chemicals administered orally come into direct contact with the forestomach epithelium, sometimes at high concentrations and for long periods. That is why chemicals when given by the oral route are more likely to give rise to forestomach tumours than when they are administered by other routes. This is especially so for gavage administration, which results in a bolus dose that exerts a traumatic effect on the forestomach epithelium. When repeated daily in chronic studies, it leads to chronic inflammation and regenerative hyperplasia. Moreover, oral gavage with a vehicle such as corn oil, which itself is a mild irritant and mitogen, results in longer retention of the compound in the forestomach than when the compound is given in water. Hence, the induction of forestomach hyperplasia and squamous cell tumours is a common finding in NTP rodent bioassay studies in which a high concentration of an irritating material suspended in corn oil is delivered by gavage into the forestomach daily for two years. For some chemicals (e.g. dichlorvos, ethyl acrylate, methyl bromide) the development of forestomach lesions has been shown to depend on administration by gavage, as opposed to other routes of administration (via food, drinking water, inhalation or skin). For other chemicals (like BHA) it has been demonstrated that when given via the diet, forestomach tumours only develop when there is continued exposure above certain dietary concentrations. This illustrates that a high local concentration at the forestomach mucosa is more important than the total body dose on a mg/kg bw basis [36].

1.4 Sensitive species/groups

1.4.1 Interspecies and sex differences

The incidence of naturally occurring benign forestomach tumours is independent of sex and is higher for hamsters than for mice and rats. Naturally occurring malignant lesions are seldomly seen in rats, mice and hamsters, and similar low non-sex-related incidences have been noted for these rodents (see section 2).

Forestomach tumours have been induced in rats, mice and/or hamsters. The majority of cases involve rats, possibly because the rat is very often the species of choice in carcinogenicity studies.
Database-analysis of 379 long-term NCI/NTP carcinogenicity studies in rats and/or mice revealed that with respect to chemical-induced forestomach carcinogenicity (as observed in 23 studies), there was a strong analogy between males and females, as well as between species. The agreement between sexes within a species (73-100% for rats, 93% for mice), however, was considerably higher than the agreement between species (approximately 55%) [21]. This picture was confirmed in a more recent analysis, including also chemicals not being part of the NCI/NTP database [22].
Despite these strong analogies, species and sex differences are possible in forestomach carcinogenesis and have indeed been observed for some chemicals. Differences in transit time and luminal conditions (pH, microflora), which are species- and gender-related, may play a role [38]. It has also been postulated that factors such as differences in microsome- or cytosol-mediated metabolic activation and covalent binding of carcinogenic chemicals to tissue macromolecules in the forestomach as well as in detoxification enzymes and DNA repair systems could be involved in species variation, while differences between the antioxidant defence systems of males and females could possibly account for some of the sex differences [13].

1.4.2 Intraspecies differences

Although the rat is very often the species of choice in carcinogenicity studies, not many different strains of rats have been used. Most studies have been conducted with F344 rats.

There is one study investigating the strain-dependence of BHA-induced rat forestomach carcinogenesis. It appeared that major strain differences exist in susceptibility to forestomach squamous cell carcinoma induction by BHA, the incidence being highest in the SHR strain, followed by SD, F344 and Lewis. In addition, there was a positive correlation between development of squamous cell carcinomas and the severity of inflammation and tissue injury. Hence, sensitivity to cytotoxicity might be an important parameter [23].

The strain-dependence of BHA-induced forestomach carcinogenesis was also investigated in two strains of Syrian golden hamsters. BHA induced moderate to severe hyperplasia and forestomach papilloma in Misaki hamsters, but failed to induce forestomach papilloma in Lakeview hamsters. In these hamsters only mild hyperkeratosis and mild hyperplasia was observed [24].

In mice, a strain-dependence was demonstrated for forestomach carcinogenesis induced by the heterocyclic amine MeIQ (2-amino-3,4-dimethylimidazo[4,5-f]quinoline): CDF1 mice were susceptible, whereas C57BL/6N mice were not [25].

Hence, strain differences in forestomach carcinogenesis are possible. However, no firm conclusions on intraspecies susceptibility are possible, because strain-dependency has been investigated only for a limited number of chemicals in a limited number of studies.

1.5 Miscellaneous / Other considerations

1.5.1 Similarities and differences of oesophagus and oesophageal region of the stomach between humans and rodents

In both rodents and humans, the oesophageal mucosa consists of stratified squamous epithelium with a keratin coating as protective barrier against possible chemical insults. The keratin coating is more pronounced in rodents than in humans. In addition to the keratin coating, humans however, in contrast to rodents, have a protective mucous layer against chemical insults. The mucus is secreted by oesophageal glands and lubricates the surface, thereby facilitating the passage of food through the oesophagus. The oesophagus of rodents does not contain mucosal glands, nor does the rodent forestomach.
In both species the muscularis provides the motive power for rapid propulsion of food material from the pharynx to the stomach. There is thus little time for contact of ingested material with the oesophageal epithelial cells, even with ‘secondary’ exposure of the oesophagus due to vomiting. Humans are capable of vomiting when sufficient irritation occurs. Rodents lack the vomiting reflex.
The oesophageal region of the stomach is the non-glandular part of the stomach lined with stratified squamous epithelium. In humans, the oesophageal region of the stomach is very limited, not to say absent. In contrast, in rodents this continuation of oesophageal epithelium (i.e. the forestomach) covers a substantial part of the stomach. The physiological conditions, however, are different between forestomach and oesophagus (a.o. pH, surface population of bacteria and yeast). Because of its storage function, the residence time of ingested material in the forestomach is much longer than in the oesophagus. Besides, when in long-term studies the

animals have free access to food, the rodent forestomach is practically exposed continuously because it is never empty [4,5].

Changes in the physiological conditions of the forestomach may also play a role in the induction of forestomach tumours by specific agents, e.g. substances stimulating the parasympathetic nervous system (like for instance cholinesterase inhibitors). Under normal circumstances, chemicals (particularly small, lipophilic substances) can be absorbed from the forestomach and, depending on the transit time and luminal pH, they can be degraded and stimulate proliferation. This process is enhanced by the presence of abundant saliva. With parasympathetic stimulation there is longer transit time, more abundant and alkaline saliva and more alkaline luminal pH. Stimulation of the parasympathetic system thus affects the luminal conditions of the forestomach, allowing the substance not only to be absorbed but, being subject to stasis, also to be degraded and to interact with epithelial cells. This enhances the potential of a chemical to produce toxicity, especially when the chemical is given by gavage, reaching the forestomach as a bolus. Over time that could result in increased proliferation and ultimately neoplasia [36].

1.5.2 Lack of effect in the oesophagus

The epithelium of the forestomach and oesophagus in the rodent are morphologically identical, they are in fact continuous, so if the forestomach squamous epithelium were to respond to an insult, then a similar response might be expected in the oesophagus[4]. However, in general in studies where forestomach tumours were induced, no oesophageal tumours were observed. Epithelial hyperplasia or other oesophageal lesions were also seldomly observed, with the exception of the occasional gavage-induced injury. This could point to a lesser sensitivity of the oesophagus, which might be explained by:
the rapid passage of ingested material through the oesophagus, and
the difference in physiological conditions between the rodent forestomach and oesophagus (as explained above).

Thus, unless a substance is extremely acidic or caustic, no chemical-cell interactions occur with the lining epithelium in the oesophagus. In contrast, in the forestomach a substance is subject to stasis and, depending on the prevailing pH, subject to degradation by microflora, allowing direct or indirect chemical-to-cell and cell-to-cell interactions [36]

1.5.3 Markers/Threshold

At an early stage prior to cancer development, strong toxicity and/or cell proliferation are caused in the rodent forestomach epithelium by many genotoxic as well as non-genotoxic forestomach carcinogens. Cytotoxicity and/or cell proliferation are therefore important markers for both carcinogenicity and/or promotion of carcinogenesis in the forestomach [13].
From experience gained at various sites in the rodent (including the forestomach) it is believed that strong proliferative non-genotoxic stimuli as the cause of tumour development have an effective threshold, unlike genotoxic substances. Below the threshold dose both the early/intermediate changes and the neoplastic changes are absent but above the threshold both

[4] Except may be for chemicals administered by gavage. In principle, administering a chemical by stomach tube circumvents direct contact of the chemical with the oesophagus. In practice, however, gavage administration may result in exposure of the distal part of the oesophagus. Especially when corn oil is used as vehicle, some of the dose may adhere to the oesophagus. Moreover, gavage administration may induce mechanical injury.

types of change are manifest. Hence, substances causing hyperplasia will only be effective in provoking tumour development when administered in doses sufficiently high to provoke proliferating stimuli. At lower doses in which no proliferative action can be found, in principle no tumours will occur. For those non-genotoxic carcinogens known to cause characteristically defined and sustainable tissue damage as a precursor of tumour development in rodents, it should thus be possible to establish a threshold dose below which both effects will not appear [11,16].

1.6 Assessment and RIVM/SIR strategy

1.6.1 Approach taken by other organisations

Several international organisations and expert committees have dealt with forestomach carcinogens. Although all conclude that it is difficult to see the relevance to humans of tumours induced in an organ which does not exist in humans, a clear guidance on what to do with the finding of forestomach tumours in animal studies with regard to the risk assessment for humans is often lacking. Some have proposed to discount certain tumours found in rodents when evaluating carcinogenic risks for humans, on the basis of mechanistic considerations (e.g. local irritant action). Rat and mouse forestomach tumours induced after oral (especially gavage) administration belong to these [30,33,34,35]. However, there is no consensus on this matter: investigators from NIEHS are of the opinion that there are few scientific data to justify routine exclusion of several site-specific tumours, including forestomach tumours [31,32].

Until recently (see below) IARC was of the opinion that rodent forestomach findings cannot be readily dismissed, but that no judgement on the potential carcinogenic hazard to man is possible on the basis of the induction of this type of tumour in rodents [1,3] For genotoxic carcinogens, even if the substance is found to be carcinogenic only in the rodent forestomach, it is generally reasoned that they cannot be regarded as safe for humans because these substances might induce irreversible, initiating events at different sites in humans [11,13].
For carcinogens inducing rodent forestomach tumours by a non-genotoxic mechanism involving cytotoxicity and/or increased cell proliferation and restorative hyperplasia, a threshold-approach is usually applied, assuming that there is no carcinogenic risk in humans as long as the exposure to humans is below the level at which tissue damage and preneoplastic lesions occur [11,16]. The threshold-approach has been proposed for several non-genotoxic forestomach carcinogens like for instance BHA [26,29; by JECFA and SCF], propionic acid [27] and chlorothalonil [28] (by several committees, like JMPR, UK-PAC, HWC). In applying the threshold-approach, mostly the NOEL for forestomach hyperplasia (if being the most sensitive toxic endpoint) is used in establishing a safe level for humans. In the case of chlorothalonil-induced forestomach lesions JMPR concluded that rodents are poor models for predicting human risk and that the dog or monkey may be more suitable models. Consequently, JMPR did not use the NOEL for forestomach hyperplasia in rodents but a NOEL from a dog study in establishing a safe level for chlorothalonil [28].

Recently, IARC addressed the mechanisms by which forestomach tumours occur in rodents and considered the predictive value of rodent forestomach tumours for the identification of carcinogenic hazards to humans [36]. IARC concluded that 'the precise underlying mechanism of action for any forestomach carcinogen is at present not fully known. Nevertheless, most genotoxic forestomach carcinogens appear to act through a mode of action involving genetic changes in oncogenes and tumour suppressing genes. Non-DNA reactive agents appear to cause forestomach tumours primarily through initial cytotoxicity and subsequent sustained cell

proliferation and hyperplasia.' IARC is of the opinion that, in principle, carcinogens targeting the forestomach squamous epithelium in rodents – even if they only cause tumours at this site – are relevant for humans, because of the following:

- while humans do not have a forestomach, they do have comparable squamous epithelial tissues in the oral cavity and (large part of) the oesophagus;
- the target tissues for carcinogens may differ between experimental animals and humans and a forestomach carcinogen in rodents may target a different tissue in humans;
- tumorigenic effects in the forestomach are usually accompanied by similar effects in other tissues, indicating that there may be either general (e.g., genotoxic or receptor interactive) or multiple modes of action.

However, IARC also recognises that the relevance for humans is probably limited for agents that have no demonstrable genotoxicity and that are solely carcinogenic for the forestomach squamous epithelium in rodents after oral administration. This because the exposure conditions during oral administration (particularly with gavage dosing) are quite different between experimental animals and humans in that physical effects may result in high local concentrations of test substances in the forestomach and prolonged exposure of the epithelial tissue. Consequently, for these agents, the mode of carcinogenic action could be specific to the experimental animals. IARC therefore recommends that in evaluating the relevance of the induction of forestomach tumours in rodents for human cancer the exposure conditions in the experiments have to be considered.

⇒ IARC-approach:

Carcinogens that cause forestomach tumours in rodents should be evaluated as if they presented a carcinogenic hazard to humans.

But:

Agents that only produce tumours in the forestomach of rodents after prolonged treatment through non-DNA reactive mechanisms may be of less relevance to humans, since human exposure to such agents would need to surpass time-integrated dose thresholds in order to elicit the carcinogenic response [36].

Unfortunately, the way this exemption is phrased ('may be of less relevance') is no firm statement on whether or not to take into account the rodent forestomach findings in human risk assessment.

1.6.2 RIVM/SIR strategy

As hypothesised in the introduction, carcinogens targeting the forestomach in rodents might induce tumours in the human oesophagus (and related organs as oral cavity and pharynx). However, from the available data it can be concluded that with respect to chemical-induced forestomach tumours, the rodent is not a suitable model for predicting effects likely to occur in humans in the upper digestive tract, in particular the oesophagus. Moreover, even in rodents forestomach carcinogens in general do not affect the oesophagus. In assessing the relevance of rodent forestomach tumours for human risk assessment one should take account of the differences in anatomy/physiology and exposure conditions between rodents and humans.

a. Differences in anatomy/physiology

- An oesophageal region in the stomach (the so-called forestomach) is present in rodents, but absent in humans.
- The rodent forestomach and oesophagus have different physiological conditions: the forestomach is colonised by microflora and subjected to changes in pH.
- Rodents lack a vomiting reflex, while vomiting enables humans to get rid of an irritating substance, which a number of forestomach carcinogens are.

b. Differences in exposure conditions
- Due to the rapid passage through the oesophagus, there will be limited or no interaction of the chemical with the epithelial lining of the oesophagus. In contrast, due to the storage function of the forestomach and the eating habits of rodents, a chemical is subject to stasis in the forestomach, allowing more interaction with the forestomach mucosa.
- In the induction of forestomach tumours by non-genotoxic carcinogens, the exposure duration and the presence of high local concentrations appear to be crucial. The exposure conditions resulting in such prolonged high local concentrations in the rodent forestomach (long-term oral administration of a chemical via gavage or in high dietary/drinking water concentrations) are not representative for human exposure conditions.

Further, it is to be noted that:
- Many forestomach carcinogens have some form of genotoxicity.
- Most genotoxic forestomach carcinogens are multi-site carcinogens.
- The mechanism of forestomach carcinogenesis by non-genotoxic substances is time- and dose-dependent and reversible (unless exposure is excessively prolonged or intense), pointing to a threshold-based mechanism: at doses below those yielding cytotoxicity and enhanced cell proliferation, there will be no promotion of tumour development.

Approach
Given the above, in establishing the risk of rodent forestomach carcinogens for humans, first a distinction has to be made between genotoxic and non-genotoxic substances, followed by a distinction in exposure route.

1. Is the substance inducing forestomach tumours in rodent studies *genotoxic*?
   a) If on the basis of the available data the answer is *yes*, then the substance should be evaluated as if it presents a carcinogenic hazard to humans. This because a genotoxic substance might induce irreversible, initiating events in related and/or different sites in humans. In the risk assessment for humans a non-threshold approach has to be applied to the rodent forestomach findings, or, if multiple sites are affected (which will almost always be the case), to the most critical site.
      Note: The database present for a given genotoxic substance may indicate that cytotoxicity rather than genotoxicity is the determining factor for forestomach tumour induction. In that case, the available data should be subjected to expert consultation, in order to decide on the primary mode of action and the way forward in risk assessment.
   b) If on the basis of the available data the answer is *no*, the exposure conditions under which the forestomach tumours were induced should be considered (question 2).

2. Are forestomach tumours induced in rodent studies *after oral administration*?
   a) If the answer is *yes*, then the effects of the substance on the rodent forestomach after oral administration are considered rodent-specific and therefore not relevant for humans. This because the exposure conditions in rodents, which are considered not representative for humans, contribute to responses that are unique for the forestomach. Hence, the rodent forestomach findings (both tumours and hyperplasia) after oral exposure do not have to be taken into account for human risk assessment. The risk assessment for humans should be based on the most critical, relevant effect identified in the available database.
   b) In the extremely rare case that the answer is *no* (given that practically all of the 'non-oral' forestomach carcinogens identified up to now are genotoxic), the available data

should be subjected to expert consultation, in order to decide on a case-by-case basis how to deal with the rodent forestomach findings in human risk assessment.

In all cases a narrative is needed to substantiate the choices made.

References

1. Takahashi, M. and R. Hasegawa (1990). Tumours of the stomach. In: Pathology of tumours in laboratory animals. Volume 1 - Tumours of the rat. IARC (WHO)/ILSI, IARC Scientific Publications No. 99, Lyon, France, 2nd ed., V.S. Turusov and U. Mohr (eds.), p.129-157.
2. Leininger, J.R. and M.P. Jokinen (1994). Tumours of the oral cavity, pharynx, oesophagus and stomach. In: Pathology of tumours in laboratory animals. Volume 2 - Tumours of the mouse. IARC (WHO)/ILSI, IARC Scientific Publications No. 111, Lyon, France, 2nd ed., V.S. Turusov and U. Mohr (eds.), p.167-193.
3. Takahashi, M. and H. Okamiya (1996). Tumours of the oral cavity, buccal pouch, oesophagus, forestomach and salivary glands. In: Pathology of tumours in laboratory animals. Volume 3 – Tumours of the hamster. IARC (WHO)/ILSI, IARC Scientific Publications No. 126, Lyon, France, 2nd ed., V.S. Turusov and U. Mohr (eds.), p.59-77.
4. Comprehensive Toxicology (1997). On CD-ROM. Elsevier Science, 1st ed., Sipes, I.G., C.A. McQueen and A.J. Gandolfi (eds.).
5. Grice, H.C. (1988) Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. Fd Chem. Toxic. 26 (8), p.717-723.
6. Fukushima, S. and N. Ito (1985a). Papilloma, forestomach, rat. In: Digestive system (Monographs on pathology of laboratory animals). T.C. Jones, U. Mohr and R.D. Hunt (eds.), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, p.289-292.
7. Fukushima, S. and N. Ito (1985b). Squamous cell carcinoma, forestomach, rat. In: Digestive system (Monographs on pathology of laboratory animals). T.C. Jones, U. Mohr and R.D. Hunt (eds.), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, p.292-295.
8. Pathology of laboratory mice and rats (1985). The Biology Databook Editorial Board. Joint project of Federation of American Societies for Experimental Biology and Pergamon Infoline Inc., Pergamon Infoline Inc., New York, USA, p.102-115.
9. Haseman, J.K., J.R. Hailey and R.W. Morris (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. Toxicol. Pathol. 26 (3), p.428-441.
10. NTP (1999). NTP Historical control information for the NIH-07 diet (website http://ehis.niehs.nih.gov/ntp/docs/ntp_hcrs.html), updated 1999/12, 2002/03.
11. Kroes, R. and P.W. Wester (1986). Forestomach carcinogens: possible mechanisms of action. Fd Chem. Toxic. 24 (10/11), p.1083-1089.
12. Wester, P.W. and R. Kroes (1988). Forestomach carcinogens: pathology and relevance to man. Toxicol. Pathol. 16 (2), p.165-171.
13. Hirose, M. and N. Ito (1999). Forestomach and glandular stomach carcinogenesis. In: Carcinogenicity. Testing, predicting, and interpreting chemical effects. K.T. Kitchin (ed.). Marcel Dekker, Inc., New York, USA, p.467-497.
14. Clayson, D.B., F. Iverson, E.A. Nera and E. Lok (1990). The significance of induced forestomach tumors. Annu. Rev. Pharmacol. Toxicol. 30, p.441-463.
15. Ghanayem, B.I., R.R. Maronpot and H.B. Matthews (1986). Association of chemically induced forestomach cell proliferation and carcinogenesis. Cancer Letters 32, p.271-278.

16. Grasso, P., M. Sharratt and A.J. Cohen (1991). Role of persistent, non-genotoxic tissue damage in rodent cancer and relevance to humans. Annu. Rev. Pharmacol. Toxicol. 31, p.253-287.
17. Kagawa, M., K. Hakoi, A. Yamamoto, M. Futakuchi and M. Hirose (1993). Comparison of reversibility of rat forestomach lesions induced by genotoxic and non-genotoxic carcinogens. Jpn. J. Cancer Res. 84, p.1120-1129.
18. Hirose, M., S. Takahashi and T. Shirai (1995). Characteristics of forestomach carcinogenesis by non-genotoxic phenolic compounds. J. Toxicol. Pathol. 8, p.277-284.
19. Ito, N., M. Hirose and S. Takahashi (1993). Cell proliferation and forestomach carcinogenesis. Environ. Health Perspect. 101 (suppl 5), p.107-110.
20. Boorman, G.A., H.L. Hong, C.W. Jameson, K. Yoshitomi and R.R. Maronpot (1986). Regression of methyl bromide-induced forestomach lesions in the rat. Toxicol. Appl. Pharmacol. 86 (1), p.131-139.
21. Haseman, J.K. and A-M. Lockhart (1993). Correlations between chemically related site-specific carcinogenic effects in long-term studies in rats and mice. Environ. Health Perspect. 101 (1), p.50-54.
22. Benigni, R. and A. Pino (1998). Profiles of chemically-induced tumors in rodents: quantitative relationships. Mutat. Res. 421, p.93-107.
23. Tamano, S., M. Hirose, H. Tanaka, A. Hagiwara and T. Shirai (1998). Variation in susceptibility to the induction of forestomach tumours by butylated hydroxyanisole among rats of different strains. Fd Chem. Toxic. 36, p.299-304.
24. Lam, L.K.T. (1988). Differential tumorigenicity of 2(3)-tert-butyl-4-hydroxyanisole in the forestomach of Lakeview and Misaki Syrian golden hamsters. Carcinogenesis 9 (9), p.1611-1616.
25. Fujita H., K. Nagano, M. Ochiai, T. Ushijima, T. Sugimura, M. Nagao and T. Matsushima (1999). Difference in target organs in carcinogenesis with a heterocyclic amine, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, in different strains of mice. Jpn. J. Cancer Res. 90 (11), p.1203-1206.
26. Whysner, J. and G.M. Williams (1996). Butylated hydroxyanisole mechanistic data and risk assessment: conditional species-specific cytotoxicity, enhanced cell proliferation, and tumor promotion. Pharmacol. Ther. 71 (1/2), p.137-151.
27. Harrison, P.T.C. (1992). Propionic acid and the phenomenon of rodent forestomach tumorigenesis: a review. Fd Chem. Toxic. 30 (4), p.333-340.
28. Wilkinson, C.F. and J.C. Killeen (1996). A mechanistic interpretation of the oncogenicity of chlorothalonil in rodents and an assessment of human relevance. Regul. Toxicol. Pharmacol. 24, p.69-84.
29. Würtzen, G. (1993). Scientific evaluation of the safety factor for the acceptable daily intake (ADI). Case study: butylated hydroxyanisole (BHA). Food Addit. Contam. 10 (3), p.307-314.
30. Davies, T.S., B.S. Lynch, A.M. Monro, I.C. Munro and E.R. Nestmann (2000). Rodent carcinogenicity tests need be no longer than 18 months: an analysis based on 210 chemicals in the IARC monographs. Fd Chem. Toxic. 38, p.219-235.
31. Karstadt, M. and J.K. Haseman (1997). Effect of discounting certain tumor types/sites on evaluations of carcinogenicity in laboratory animals. Am. J. Ind. Med. 31, p.485-494.
32. Haseman, J., R. Melnick, L. Tomatis and J. Huff (2001). Carcinogenesis bioassays: study duration and biological relevance. Fd Chem. Toxic. 39, p.739-744.
33. Commission on Risk Assessment and Risk Management (CRARM) (1996). Risk assessment and risk management in regulatory decision-making. Draft report for public review and comment. Washington D.C., 23-26, Table 3.2. [As cited in Karstadt and Haseman, 1997; original publication not available.]

34. Environmental Protection Agency (EPA) (1996). Proposed guidelines for carcinogen risk assessment, notice of availability and opportunity to comment on proposed guidelines for carcinogen risk assessment. Fed. Reg. 61, p.17959-18011 (April 23). [As cited in Karstadt and Haseman, 1997; original publication not available.]
35. Environmental Health Letter (EHL) (1996). Irrelevant rodent tumors should not influence human risk assessments. (May 6), p.85-86. [As cited in Karstadt and Haseman, 1997; original publication not available.]
36. IARC (2003). Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans. Views and expert opinions of an IARC Working Group, Lyon, 29 November – 1 December 1999. IARC Technical Publication No. 39, Lyon, France, p.1-73.
37. NTP (2003). NTP Historical controls for the NTP-2000 diet (website http://ntp-server.niehs.nih.gov/Main_Pages/ntp_hcrs.html), updated 2003/02.
38. Iatropoulos, M.J. (1993). Comparative histokinetic and xenodynamic considerations in toxicity. In: Drug toxicokinetics. P.G. Welling and F.A. de la Iglesia (eds.), Marcel Dekker, New York, p.245-266. [As cited in IARC, 2003; original publication not available.]

2. Leydig cell tumours

Factsheet FSV-012/00, date 16-12-2003

Author: S.M.J.G. Pelgrom

Contents

2.1 Introduction and aim 24
2.2 General information and mechanisms of induction 24
2.2.1 Direct DNA reactive mechanisms 27
2.2.2 Non-DNA reactive mechanisms 27
2.2.3 Other possible mechanisms 28
2.3 Normal values and natural variation 28
2.4 Sensitive species/groups 29
2.4.1 Interspecies 29
2.4.2 Intraspecies 30
2.5 Assessment and RIVM/SIR strategy 30
References 32
Annex I 34
Annex II 39

2.1 Introduction and aim

Spontaneous testicular tumours are commonly observed in some rat strains [2,3,21] and in dogs [20] and mice [6,7]. The tumours can be divided in three types: Sertoli cell tumour, seminoma and Leydig cell tumour (interstitial cell tumour) [9,20]. The main function of Leydig cells (LCs) is the production of testosterone.
Leydig cell tumours (LCTs) are mostly benign and are observed especially in older animals [2,20,21].
Some uncertainty exists about the true occurrence of Leydig cell adenomas in man, although occurrence seems to be rare and restricted primarily to white males [7]. The known risk factors for LC adenomas are restricted to heritable disorders of the endocrine system (congenital adrenal hyperplasia and androgen insensitivity syndrome), Klinefelter's syndrome, Androgen Insensitivity Syndrome (AIS) [Quigly et al. 1995 *in* 25], familial male precocious puberty (FMPP) [Shenker et al. 1993, 1995 *in* 25] and cryptorchidism with related testicular atrophy [21,23, ref. 104 in 7].
LCs are a common target of compounds tested in rodent carcinogenicity bioassays [9]. Doubts have been raised about the relevance of chemical-induced LCTs for human risk assessment [1; 2,7,9,21]. In this factsheet, the toxicological relevance of a chemical-induced increase in LCTs in laboratory animals for human risk assessment will be discussed.

2.2 General information and mechanisms of induction

In the rat the distinction between LC hyperplasia and adenoma (tumour) is, like other endocrine tumours in the rat, arbitrarily based on size [7-9]. There has been much discussion concerning the exact size that should be used to discriminate between hyperplasia and adenoma. Two arbitrary criteria have been developed for rodents [9]:

- According to the National Toxicology Program (NTP), an aggregate of LCs smaller than the diameter of a seminiferous tubule is classified as being focal hyperplasia [Boorman et al. *in* 9]. A mass of LCs greater than that of a seminiferous tubule is classified as a tumour. Masses of this size generally produce some compression of adjacent tubules.
- Guidelines recommended by The Society of Toxicologic Pathologists for standarization of diagnosis propose three tubular diameters as the arbitrary separation of focal LC hyperplasia from LC neoplasia [McConell et al. *in* 9]. In addition, the proliferative focus must also have morphological features consistent with LCT. These features may include evidence of autonomous growth by symmetrical peripheral compression of adjacent seminiferous tubules, evidence of cellular pleomorphism, and development of a typical endocrine sinusoidal vascular network.

To date, the debate continues without a generally accepted size criterium [9].
In toxicology safety assessment studies, almost none of the references use the pathological criteria to distinguish between LC hyperplasia (LCH) and LCT [9]. It is considered that the transition from LCH to LCT is part of a continuous spectrum of change, and both LCHs and LCTs may be routinely pooled in the interpretation and analysis of carcinogenicity studies [21]. In contrast to rodents, no size criteria is used to distinguish between hyperplasia and tumour in humans [Mostofi and Davis, 1990 *in* 9].

A number of chemicals, including many non-DNA-reactive compounds, have been shown to increase the incidence of LC hyperplasia and tumours in chronic studies in certain strains of rats, and occasionally in mice and dogs [21]. Morphologically, there appears to be no difference between spontaneous and chemically induced LCTs [8; 21].

For many of the compounds that induce LCH and/or LCT the mechanism of action is not known. However, there are some generalisations that can be made regarding the mode of action or chemical activity, primarily involving effects on the hypothalamic-pituitary-testicular axis (HPT-axis).

The regulation of testosterone production by LCs can be divided into extratesticular (via pituitary) and intratesticular (paracrine: by seminiferous tubules, and autocrine: by LC products such as estradiol and testosterone) [14]. The primary sites in the regulation of testosterone production in rats and humans are the hypothalamus and the pituitary (see Figure 1) [9].
In order to understand how chemicals may induce LCTs by interrupting the HPT-axis, it is necessary to understand the regulation of the HPT-axis. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the secretion of luteinizing hormone (LH) by the pituitary. LH maintains testosterone levels in homeostasis [ref 17 *in* 8,9,15,21] and LH binds to Leydig cells and activates adenylate cyclase to increase cAMP levels. Increased cAMP levels stimulate testosterone biosynthesis causing testosterone levels to rise in the bloodstream. Testosterone exerts a negative feedback on the release of GnRH and LH from the hypothalamus and pituitary, respectively (see fig. 1) [8,9].
An additional feedback control mechanism of LH involves the steroid estradiol. Testosterone is converted to estradiol via the enzyme aromatase, a process which is commonly referred to as aromatization. Aromatase activity is found in adipose tissue, liver, skeletal muscle and testis. In males, the majority of estradiol synthesis occurs in adipose tissue. Similarly to testosterone, increasing blood estradiol levels will attenuate LH secretion.

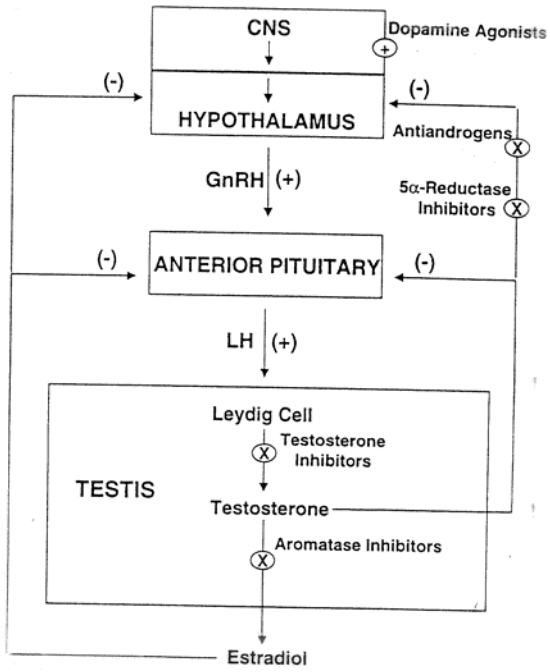

Figure 1. Regulation of the HPT-axis and control points for potential disruption. Symbols: (+) feedback stimulation; (-) feedback inhibition; ⊕ receptor stimulation; ⊗ enzyme or receptor inhibition [8,9].

Figure 1 also illustrates mechanisms by which chemicals can disrupt the HPT-axis and produce LCTs in rats. In animals treated with some LC tumour-inducing agents, an increased level of circulating LH was observed, leading to the current concept that these adenomas arise due to chronic LH elevation [2,7,8].

Increases in LH level were not seen in all studies of chemicals for which the proposed mode of action calls for elevated LH. Compensation may occur to restore homeostasis, such as induction secondary to seminiferous tubule damage, paracrine involvement, events related to peroxisome proliferation and increased steroid clearance due to enzyme induction [7].

In tables 1 and 2 (see Annex I) compounds are listed that produce LC hyperplasia and/or LCTs in rats, mice or dogs. Because of the implications in risk assessment, DNA-reactive and non-DNA-reactive compounds are considered separately [7].

2.2.1 Direct DNA reactive mechanisms

Most of the genotoxic compounds that induce LCTs also induce adenomas and/or carcinomas at other sites (see table 2 in Annex I; [9]), and LCT is one of the endpoints to be considered [7].

2.2.2 Non-DNA reactive mechanisms

The non-genotoxic compounds are subdivided by their mode of action, chemical activity, chemical class or as other (table 1 in Annex I); [9].
There are 7 modes of action that appear to affect hormonal control of LC activity [7,9].
Androgen receptor antagonism: competition with testosterone and dihydrotestosterone (DHT) for binding to the androgen receptor. This competition reduces the net androgenic signal to the hypothalamus and pituitary resulting in an increase in LH with a concomitant elevation of testosterone.

1. Testosterone biosynthesis inhibition: decrease in testosterone levels increases LH levels in rats, resulting in the development of LCTs.
2. 5α-Reductase inhibition: blocks the conversion of testosterone to DHT. DHT amplifies the androgenic signal through several mechanisms: (i) unlike testosterone, DHT cannot be aromatized to estrogen and thus its effect is purely androgenic and (ii) DHT binds to the androgenic receptor with greater affinity and stability than testosterone. Hence, 5α-reductase inhibitors decrease DHT levels, which reduce the net androgenic signal received by the hypothalamus and pituitary and thereby causes a compensatory increase in LH levels. 5α-reductase inhibitors induce LCTs in mice and LC hyperplasia in rats.
3. Aromatase inhibition: blocks the conversion of testosterone to estradiol, resulting in a decrease in estradiol and an increase in LH levels. (In some chronic studies, dogs have been reported to be more sensitive than rats for the development of LC hyperplasia in response to aromatase inhibition [7,24].
4. Estrogen agonism: induces LCTs in certain strains of mice (Alderly Park outbred, BALB/c, Strong A) but not in rats [8]. These strain differences have been attributed to 2 factors: (i) estrogen increase LH levels in mice, but decrease LH levels in rats and (ii) estrogen may directly stimulate LC proliferation via a paracrine mechanism [7,9].
5. GnRH agonism: the only documented examples of a non-LH type mechanism that can induce LCTs. GnRH agonists induce LCTs in rats by binding to GnRH (or LHRH, luteinizing hormone releasing hormone) receptors on Leydig cells. Because Leydig cells from mice, monkey and human do not contain GnRH (LHRH) receptors, these species are believed to be not susceptible to tumour induction by this class of compounds [7,8,10,16,21].
6. Dopamine agonism: decreases serum prolactin levels in rats, which causes downregulation of LH receptors on LCs, and thus a decrease in testosterone production. Decreased testosterone results in an increased LH level. This effect has not been reported for any other species than rat (and decreased prolactin does not decrease the number of LH

receptors on human LC). An alternative mechanism has been proposed, namely that dopamine agonists increase GnRH levels that subsequently increases LH levels. The relative contribution of these two mechanisms toward the development of LCTs remains to be determined [9].

2.2.3 Other possible mechanisms

Since many compounds are able to induce LC hyperplasia and/or tumours by mechanisms other than the HPT-axis disrupting mechanisms described above, many studies have focussed on additional modes of action for LCT induction, although the contribution of the suggested mechanisms needs to be elucidated. Additional factors with LCT-inducing potential are discussed below.

- In addition to the direct effects of elevated levels of LH, proto-oncogene activation appears to be also a consequence of LH stimulation of LC, and might provide a common underlying mechanism [7].
- Testosterone production is also suppressed by glucocorticoids in rats (elevated after stress) [9]. It is proposed that stress (indicated by an increase in serum corticosteroid levels), related to individual caging, particular among males, directly impairs testosterone synthesis and produce LC atrophy, which leads to a feedback increase in the synthesis of LH by the anterior pituitary [19]. This phenomenon has been observed in toxicity studies with rats. (note: rats in inhalation and dermal toxicity studies are singly caged, whereas in feed or gavage studies, rats are group-caged [19].
- While it is clear that normal functioning of the LCs is dependent on an appropriate endocrine environment within the testis, primarily that provided by LH stimulation, the paracrine environment also plays an important role (seminiferous damage, IGF-1, TGFα, IL-1) [9,14,21].
- Several peroxisome proliferators have been shown to induce LCTs in rats, without inducing peroxisomes in the Leydig cells (whereas peroxisome proliferation was observed in the liver) [7,8].
- Besides cAMP, other second messenger systems (arachidonic acid, leukotrienes, calcium/calmoduline, chloride ions, free radicals [ref. 28 *in* 7,9] may also be involved the induction of LC hyperplasia [7].

2.3 Normal values and natural variation

The spontaneous incidence of LCTs in animals is species, strain and age dependent [5,7-9]. See tables 3 and 4 of Annex II.
The comparison of spontaneous incidences between animal and man is tenuous because 1) the diagnosis in animals is from histological examination from animal experiments (standard observation) while in man (with complaint) from palpation, and 2) some rat strains are (very) sensitive (high LCT incidences). Inspite of these differences, the data suggest a substantial lower occurrence of LCT in humans compared to rats [7].

Rats
In Sprague-Dawley, Osborne-Mendel and Brown-Norway rats, LCTs are generally very rare, whereas their incidence reaches 90-100% in 18-24 month-old F344 rats. Wistar-derived strains exhibit variable incidences ranging from less than 10% to nearly 100% [2,6,7]. Histologically, about 90% of LCTs in rats are considered to be benign [5,21]. In the remaining 10% malignant tumours, distant organ metastasis is extremely rare [5].

Mice
Spontaneous incidence of LCTs in mice older than 18 months is considerably lower than in rats, and ranges from 0.1 to 2.5% [4, ref. 4 and 5 in 7].

Dogs
The spontaneous incidence of LCTs in beagle dogs at 7.75 years is 6.3%, and a further 8% showed Leydig cell hyperplasia [9,17], whereas the reported incidence for dogs in general ranges from 0.034% to 16%. In dogs, the tumours are mostly benign, and metastases are observed in only 2% [20].

Humans
The estimated incidence of LCTs is 0.3 to 3 per million [2,9]. In man, LCTs are distributed equally in various age groups [21,23]. The incidence of LCTs appears to vary by ethnic background, where the highest incidence is seen in white males [9,23]. An important risk factor is cryptorchidism, which occurs in greater frequency in white men [3,23]. Prenatal exposure to estrogens, specifically DES, has been shown to be a risk factor for cryptorchidism although not in all studies [3].
Children with LCT are more likely to demonstrate a definite clinical syndrome and may develop isosexual precocious pseudopuberty or feminization. Adult men may show feminization but often demonstrate no symptoms other than a testicular mass. Children with LCTs usually show a high urinary excretion of 17-ketosteroids, while adults have normal values, unless the tumour in the adult is metastatic. Probably all feminizing LCTs produce estrogens (high urinary estrogen excretion and high spermatic vein estradiol to testosterone ratio) [15].

A summary is given in Table 1 below.

Table 1. LCT incidence in different species

Species	LCT incidence (%)
Rats	0.8 - 100
Mice	0.1 – 2.5
Dogs	0.03 - 16
Humans	$0.3 \times 10^{-6} – 3 \times 10^{-6}$

2.4 Sensitive species/groups

2.4.1 Interspecies

Although in literature high incidences of natural occurring LCTs are described for both rats and dogs, studies on mechanisms of LCT induction are usually restricted to rats (and mice), and also most carcinogenity studies are performed with rodents. Therefore, most information is available on rats. Information on interspecies differences are mainly restricted to rat and human.
Although the anatomy and regulation of the HPT-axis are generally comparable between rats, mice, dogs, monkeys and humans, there are some differences, which may play a role in the differences in sensitivity of induction of LCTs in these species. The interspecies differences include:

- Rats lack sex hormone binding globulin (SHBG). In man, ~95% of testosterone in peripheral blood is bound to SHBG, which retards its metabolism and clearance [9]. Because the ratio between bound and free (bioavailable) testosterone is kept in balance, it is relatively difficult in man to perturb the peripheral levels of testosterone in any short-term way. In contrast to man, the rat has no peripheral SHBG and thus the blood levels of testosterone can potentially be altered more rapidly [9]. The half life of circulating LH in humans is in excess of 100 minutes, while in the rat the half life is 5 to 10 minutes [9].
- Rats have a greater LH receptor number per Leydig cell than humans (human LCs contain approximately 1500 LH receptors/cell, whereas rat LCs contain approximately 20000 receptors/cell, a 13-fold difference between rats and humans) [7,9,21].
- Rats have GnRH receptors on their LCs, in contrast to mice, monkeys and humans [7,21].
- Prolactin modulates LH receptor levels on LCs in rats, but not in humans [7].
- Rats and humans appear to respond different to exogenous hCG (human Chorionic Gonadotropin, a hormone equivalent to LH in its action on LCs), with rat LC showing hyperplasia and human LC showing hypertrophy [7,21].
- Mice and monkey appear to be less sensitive than rats to androgen receptor antagonists. There is no information available for humans [7].
- 5α-reductase inhibitors induce LC hyperplasia in rats and LC hyperplasia and LCTs in mice. There is no information available for humans [7].
- Estrogen agonists induce LCTs in mice by mechanisms that do not appear to be present in rats. No information is available for humans [7].
- Histologic and electron microscopic features of malignant LCTs in rats resemble that of human LCT, except for the presence of crystalloids of Reinke which are present in 40% of the human LCTs, and are not observed in rats [5,7]. The function of Reinke's crystals is unknown [18]. The presence of Reinke crystals is unique for human LC and the Australian wild bush rat [9].
- Testosterone levels decline with age in most strains of rats as well as in humans. In rats, this decrease is probably secondary to declining LH levels. This is in contrast to the situation in man, where LH levels tend to increase with age, presumably related to decreasing testosterone levels [9].

All of these differences may contribute to the observed higher incidence (and probably greater sensitivity for induction) of LCH/LCT in rodents when compared with human.

2.4.2 Intraspecies

Apart from strain differences in the rat and the observed higher incidence of LCTs in older dogs (in absence on information in the literature on the mechanisms of induction of LCTs in older dogs), there are no indications for specific sensitive groups within a species.
Human variation is described in Section 2.3.

2.5 Assessment and RIVM/SIR strategy

Known human incidence of clinically detectable LCTs in the general population is very low. However, occurrence of LC hyperplasia is unlikely to be detected in men of any age since routine autopsy typically does not include microscopic evaluation of the testes [7,9]. The true occurrence of LCTs in humans may be higher than currently thought, but is nonetheless significantly lower than in rats [9,21].
The pathways for the regulation of the HPT-axis of rat and humans are qualitatively similar. It appears that there is evidence that suggest that human LCs are quantitatively less sensitive than

rats in their proliferate response to LH, and hence their sensitivity to chemically induced LCTs [9]. Nevertheless, the available evidence for most mechanisms of action is insufficient to conclude that they are not relevant for humans. For LCT induction there are, based on the current state of knowledge, only 2 mechanisms for which it can be concluded that they are not relevant to humans: GnRH and dopamine agonists. Because testicular GnRH and Prolactin receptors are either not expressed or expressed only at a very low level in humans, the induction of LCTs in rats by GnRH and dopamine agonists would appear to be not relevant to humans. However, the relevance to humans of the remaining 5 mechanisms of action cannot be ruled out [9].

Generally, only in cases where a species-specific mechanism is involved in the induction of LCTs, and there is sufficient evidence that the mechanism of LCT induction is not relevant in humans, the increases in LCTs in the testspecies are not considered in the risk assessment. In circumstances in which the mechanism of induction is unknown, it should be assumed that humans are potentially susceptible [7]. This view is in line with the view of the EPA and Classification and Labelling on LC hyperplasia/neoplasia [11-13,22,25].

The distinction between LC hyperplasia and LC tumour is based on size. There are no uniform used/accepted size criteria although the criteria of the Society of Toxicologic Pathologists are frequently used. Since it is considered that the transition from LC hyperplasia to LCT is a continuous spectrum of change, both hyperplasia and neoplasia are considered in the risk assessment. For the risk assessment, the relevance of increased LCTs in laboratory animals is considered separately for genotoxic and non-genotoxic compounds.

1. Only in cases where a species-specific mechanism is involved in the induction of LCTs, and there is sufficient evidence that the mechanism of LC induction is not relevant in humans (in rat GnRH and dopamine agonists), the compound inducing the tumour is considered as carcinogenic for the species concerned, but not for humans (see above).
2. Genotoxic compounds
   Most of the genotoxic compounds that induce LCTs also induce adenomas and/or carcinomas at other sites and LCT is one of the endpoints to be considered in the risk assessment. Even when only LCT are observed, it cannot be excluded that these substances induce tumours at other sites in humans. Therefore, the LCTs are considered to be relevant for human risk assessment.
3. Non-genotoxic compounds
   An increase in LCT number has to be statistically significant and dose-related for considering in the risk assessment as evidence of carcinogenicity. A statistically significant increase may also occur in the high dose only.
   Accompanying LC hyperplasia contributes to the weight of evidence of the neoplastic response. However, a compound is not designated carcinogenic based on increased hyperplasia only.

Expert consultation is needed:
- when the increase in LC hyperplasia/neoplasia is the critical effect for determining the NOAEL of a study.
- when a non-significant increased incidence in LCTs, dose-related and/or accompanied by hyperplasia, is observed in species/species strains, which have a low spontaneous incidence of LCTs (see Annex II) .
- when only a significantly (dose-related) increased incidence of hyperplasia is observed.

- when the LCT incidence in the control group deviates from the normal background incidence in that species/species strain, and the LCT numbers observed in the dosed groups may pose a problem.

References

1. Alison, R. H.; Capen, C. C., and Prentice, D. E. Neoplastic lesions of questionable significance to humans. Toxicologic Pathology. 1994; 22((2)):179-186.
2. Bär, A. Significance of Leydig cell neoplasia in rats fed lactitol or lactose. Journal of the American College of Toxicology. 1992; 11((2)):189-207.
3. Bosland, M. C. Hormonal factors in carcinogenesis of the prostate and testis in humans and animal models. Prog. Clin. Biol. Res. 1996; 394:309-352.
4. Chandra, M. and Frith, C. H. Spontaneous neoplasms in aged CD-1 mice. Toxicology Letters. 1992; 61:67-74.
5. Chandra, M. and Riley, M. G. I. Rarely occurring spontaneous metastasizing testicular tumors in rats. Histopathologic and ultrastructural features. Exp. Toxic. Pathol. 1994; 46:155-161.
6. Chandra, M.; Riley, M. G. I., and Johnson, D. E. Spontaneous neoplasms in aged sprague-dawley rats. Arch. Toxicol. 1992; 66:496-502.
7. Clegg, E. D.; Cook, J. C.; Chapin, R. E.; Foster, P. M. D., and Daston, G. P. Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reproductive Toxicology. 1997; 11(no 1):107-121.
8. Cook, J. C.; Frame, S. R., and Obourn, J. D. Leydig cell tumors. Sipes, I. G.; McQueen, Ch. A., and Gandolfi, A. Comprehensive Toxicology. volume 10 ed.
   Notes: http://127.0.0.1/toc/TOC1014.HTM
9. Cook, J. C.; Klinefelter, G. R.; Hardisty, J. F.; Sharpe, R. M., and Foster, P. M. D. Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. Critical Reviews in Toxicology. 1999; 29((2)):169-261.
10. Donaubauer, H. H.; Kramer, M.; Krieg, K.; Mayer, D.; Von Rechenberg, W.; Sandow, J., and Schütz, E. Investigations of the carcinogenicity of the LH-RH analog Buserelin (HOE 766) in rats using the subcutaneous route of administration. Fundamental and Applied Toxicology. 1987; 9:738-752.
11. EPA, Federal Register. Part IV 40 CFR Parts 180 and 185. Revocation of pesticide food additive regulations; final rule.C. Iprodione. [Web Page]. 1996 Jul 29.
    Notes: Fedreal Register/ Vol. 61, no. 146/ Rules and Regulation, pp39527-39540
12. EPA, Pesticide Fact Sheet. Tralkoxydim, conditional registration. [Web Page]. Accessed 1998 Dec 4.
13. EPA Reregistration Eligibility Decision (RED). Iprodione [Web Page]. 1998 Nov.
    Notes: EPA738-R-98-019
14. Ewing, L. L. The Leydig cell. Scialli, A. R. and Clegg, E. D. Reversibility in testicular toxicity assessment. CRCPress, Boca Raton, FL; 1992: 87-126.
15. Freeman, D. A. Steroid hormone-producing tumors of the adrenal, ovary, and testes. Endocrinol. Metab. Clin. North Am.. 1991; 20:751-766.
16. Hunter, M. G.; Sullivan, M. H. F.; Dix, C. J.; Aldred, L. F., and Cooke, B. A. Stimulation and inhibition by LHRH analogues of cultured rat Leydig cell function and lack of effect on mouse Leydig cells. Molecular and Cellular Endocrinology. 1982; 27:31-44.
17. James, R. W. and Heywood, R. Age-related variations in the testes and prostate of beagle dogs. Toxicology. 1979; 12:273-279.
18. Naughton, C. K.; Nadler, R. B.; Basler, J. W., and Humphrey, P. A. Leydig cell hyperplasia. Review. British Journal of Urology. 1998; 81:282-289.

19. Nyska, A.; Leininger, J. R.; Maronpot, R. R.; Haseman, J. K., and Hailey, J. R. Effect of individual versus group caging on the incidence of pituitary and Leydig cell tumors in F344 rats: proposed mechanism. Medical Hypotheses. 1998; 50:525-529.
20. Peters, M. A. J. and Sluijs, F. J. Testistumoren bij de hond: een literatuuroverzicht. Tijdschrift Voor Diergeneeskunde. 1996; 121((2)):36-38.
21. Prentice, D. E. and Meikle, A. W. A review of drug-induced Leydig cell hyperplasia and neoplasia in the rat and some comparison with man. Human and Experimental Toxicology. 1995; 14:562-572.
22. Risk Assessment Forum U.S. Environmental Protection Agency. Special report on environmental endocrine disruption: an effects assessment and analysis. [Web Page]. 1997 Feb. Available at: http://www.epa.gov//reg5ogis/risk/endocrin.pdf.
Notes: EPA/630/R-96/012
23. Schottenfeld, D.; Warshauer, M. E.; Sherlock, S.; Zauber, A. G.; Leder, M., and Payne, R. The epidemiology of testicular cancer in young adults. American Journal of Epidemiology. 1980; 112((2)):232-246.
24. Walker, U. J. and Nogués, V. Changes induced by treatment with aromatase inhibitors in testicular Leydig cells of rats and dogs. Exp. Toxic. Pathol. 1994; 46:211-213.
25. ECBI/61/03. A “Thought Starter” for developing an agreed position on the relevance of leydig cell tumours in rats to humans. Draft Document UK, oct. 2003.

Annex I

Nongenotoxic Compounds that Produce LC Hyperplasia or Adenomas in Rats, Mice, or Dogs

Compound (CAS number)	Species (strain)*	LC response	Other tumor sites	Ref.
A. Classified by Mode of Action				
1. Androgen receptor antagonists				
Bicalutamide (90357-06-5)	Rat (Wistar)	Adenoma	Thyroid	Iswaran et al., 1998
Cimetidine (51481-61-9)	Rat (Wistar)	Adenoma	None	Leslie et al., 1981; Sivelle et al., 1982, Brimblecombe and Leslie, 1984
Fenvalerate (51630-58-1)	Rat (SD)	Adenoma (equivocal)	None	Parker et al., 1984; Eil and Nisula, 1990
Flutamide (13311-84-7)	Rat	Adenoma	None	PDR, 1995d; Viguier-Martinez et al., 1983a,b; Cook et al., 1993
Linuron (330-55-2)	Rat (CD)	Adenoma	None	Cook et al., 1993
Procymidone (32809-16-8)	Rat (Osborne-Mendel)	Adenoma	None	Hosokawa et al., 1993a,b; Murakami et al., 1995
Vinclozolin (50471-44-8)	Rat	Adenoma	None	Gray et al., 1994; Wong et al., 1995
Zanoterone (107000-34-0)	Dog (Beagle)	Hyperplasia	None	Juniewicz et al., 1990
2. 5α -Reductase inhibitors				
Finasteride (98319-26-7)	Rat (CD)	Hyperplasia	None	PDR, 1995m; Prahalada et al., 1994, George et al., 1989
	Mouse (CD-1)	Adenoma	None	PDR, 1995m; Prahalada et al., 1994
3. Testosterone biosythesis inhibitors				
Calcium Channel Blockers (see Section B)	Rat	Adenoma	None	PDR, 1995c,i,l; Roberts et al., 1989
Cimetidine (51481-61-9)	Rat (Wistar)	Adenoma (equivocal)	None	Leslie et al., 1981; Brimblecombe and Leslie, 1984; Morita et al., 1990
Ethanol (64-17-5)	Rat (CD)	Adenoma (equivocal)	None	Cheever et al., 1990; Widenius et al., 1989
Lansoprazole (103577-45-3)	Rat (CD)	Adenoma	None	Fort et al., 1995; Meikle et al., 1994
Lead acetate (301-04-2/15347-57-6)	Rat (Wistar)	Adenoma (equivocal)	None	Zawirska and Medras, 1968
Metronidazole (443-48-1)	Rat (Wistar)	Adenoma	Pituitary	Rustia and Shubik, 1979,
Vinclozolin (50471-44-8)	Rat	Adenoma	None	Ronis et al., 1994; Gray et al., 1994; Wong et al., 1995
4. Aromatase inhibitors				
Formestane (566-48-3)	Dog (Beagle)	Hyperplasia	None	Junker-Walker and Nogues, 1994
Letrozole (112809-51-5)	Dog (Beagle)	Hyperplasia	None	Junker-Walker and Nogues, 1994
5. Dopamine agonists/enhancement of dopamine levels				
Mesulergine (64795-35-3)	Rat (Wistar)	Adenoma	None	Prentice et al., 1992; Dirami et al., 1996
Norprolac (87056-78-8)	Rat (CD)	Adenoma	None	Roberts et al., 1993
Oxolinic acid (14698-29-4)	Rat (Wistar)	Adenoma	None	Yamada et al., 1994a,b; Yamada et al., 1995a,b
6. GnRH agonists				
Buserelin (57982-77-1)	Rat (Wistar)	Hyperplasia	None	Donaubauer et al., 1987
Histrelin (76712-82-8)	Rat	Adenoma	Pituitary	PDR, 1995o
Leuprolide (74381-53-6)	Rat	Adenoma	Pituitary	PDR, 1995h
Nafarelin (76932-56-4)	Rat	Adenoma	Pituitary	PDR, 1995p

(continued)

Nongenotoxic Compounds that Produce LC Hyperplasia or Adenomas in Rats, Mice, or Dogs

Compound (CAS number)	Species (strain)[a]	LC response	Other tumor sites	Ref.
A. Classified by Mode of Action (continued)				
7. Estrogen agonists/antagonists				
Diethylstilbestrol (56-53-1)	Mouse (BALB/c)	Adenoma	None	Baroni et al., 1966; Huseby, 1976
Estradiol (50-28-2)	Mouse (BALB/c)	Adenoma	None	Huseby, 1980; Nishizawa et al., 1988
Ethinylestradiol (57-63-6)	Mouse (ICR)	Hyperplasia	None	Yasuda et al., 1986; Yasuda et al., 1988
Methoxychlor (72-43-5)	Mouse (BALB/c)	Adenoma	None	Reuber, 1979
Sigetin (60252-39-3)	Rat (Strong A)	Adenoma	None	Ird, 1983
Tri-*p*-anisyl-chloroethylene (TACE) (569-57-3)	Mouse (C57x CBA)	Adenoma	Liver	Gardner and Boddaert, 1950
Tamoxifen (10540-29-1)	Mouse (Alderley Park)	Adenoma	None	Tucker et al., 1984
Triphenylethylene (58-72-0)	Mouse (Strong A)	Adenoma	None	Bonser, 1942
B. Grouped by Chemical Activity				
1. Antihypertensives				
Guanadrel (22195-34-2)	Rat	Adenoma	None	PDR, 1995f
Hydralazine (86-54-4)	Rat	Adenoma	Liver	PDR, 1995a
2. Calcium channel blockers				
Felodipine (72509-76-3)	Rat	Adenoma	None	PDR, 1995i
Isradipine (75695-93-1)	Rat (CD)	Adenoma	None	Roberts et al., 1989; PDR, 1995c
Lacidipine (103890-78-4)	Rat (CD)	Adenoma	None	Hamada and Futamura, 1998
Nimodipine (66085-59-4)	Rat (Wistar)	Adenoma	None	PDR, 1995i
3. Fungicides				
Procymidone (32809-16-8)	Rat (Osborne-Mendel)	Adenoma	None	Hosokawa et al., 1993a,b; Murakami et al., 1995
Vinclozolin (50471-44-8)	Rat	Adenoma	None	Wong et al., 1995, Ronis et al., 1994
Folpet (133-07-3)	Rat (CD)	Adenoma	Thyroid	Quest et al., 1993
4. Goitrogens				
Ethylenethiourea (ETU) (96-45-7)	Rat	Adenoma (equivocal)	None	Gak et al., 1976
6-*n*-Propyl-2-thiouracil (PTU) (51-52-5)	Rat (SD)	Hyperplasia	None	Mendis-Handagama and Sharma, 1994

(continued)

Nongenotoxic Compounds that Produce LC Hyperplasia or Adenomas in Rats, Mice, or Dogs

Compound (CAS number)	Species (strain)[a]	Leydig cell response	Other tumor sites	Ref.
B. Grouped by Chemical Activity (continued)				
5. Peroxisome proliferators				
Ammonium Perfluorooctanoate (C8) (3825-26-1)	Rat (CD)	Adenoma	Liver pancreas	Sibinski, 1987; Cook et al., 1992; Cook et al., 1994
Clofibrate (637-07-0)	Rat (Alderley Park)	Adenoma	Liver pancreas	PDR, 1995b
Diethylhexylphthalate (DEHP) (117-81-7)	Rat (SD)	Adenoma	None	Berger, 1995
Gemfibrozil (25812-30-0)	Rat (CD)	Adenoma	Liver pancreas	Fitzgerald et al., 1981
HCFC-123 (306-83-2)	Rat (CD)	Adenoma	Liver pancreas	Malley et al., 1995
Methylclofenapate (21340-68-1)	Rat (Alderley Park)	Adenoma	Liver pancreas	Tucker and Orton, 1995
Perchloroethylene (PCE) (127-18-4)	Rat (F344)	Adenoma (equivocal)	Leukemia kidney skin	Clarke and Ragan, 1986
Trichloroethylene (TCE) (79-01-6)	Rat (SD)	Adenoma	Leukemia kidney	Malloni et al., 1988
Wyeth-14,643 (50892-23-4)	Rat (CD)	Adenoma	Liver pancreas	Cook et al., 1994

Compound (CAS number)	Species (strain)[a]	LC response	Other tumor sites	Ref.
C. Grouped by Chemical Class				
1. Fluorochemicals				
HCFC-123 (306-83-2)	Rat (CD)	Adenoma	Liver pancreas	Malley et al., 1995
HCFC-133a (75-88-7)	Rat (Wistar)	Adenoma	None	Longstaff et al., 1984
HFC-134a (811-97-2)	Rat (Wistar)	Adenoma	None	Collins et al., 1995
HCFC-141b (1717-00-6)	Rat (CD)	Adenoma	None	Turnbull et al., 1994
2. Nitroaromatics and related compounds				
p-Nitrochlorobenzene (100-00-5)	Rat (SD)	Adenoma	None	Schroeder and Daly, 1984
Nitroglycerine (55-63-0)	Rat (CD)	Adenoma	Liver	Ellis et al., 1984; PDR, 1995j
2,4-Toluenediamine (95-80-7)	Rat (F344)	Adenoma	Liver pancreas	Cardy, 1979
3. Organochlorines				
o,p'-DDD	Rat	Adenoma (equivocal)	None	Lacassagne and Hurst, 1965
o,p'-DDT (789-02-6)	Rat (Osborne-Mendel)	Adenoma (equivocal)	Liver	Fitzhugh and Nelson, 1947
4. Sugars				
Lactose (63-42-3)	Rat (Wistar)	Adenoma	None	Sinkeldam et al., 1992
Lactitol (585-86-4)	Rat (Wistar)	Adenoma	None	Sinkeldam et al., 1992
Tara gum	Rat (F344)	Adenoma (equivocal)	None	Melnick et al., 1983

(continued)
Nongenotoxic Compounds that Produce LC Hyperplasia or Adenomas in Rats, Mice, or Dogs

Compound (CAS number)	Species (strain)*	LC response	Other tumor sites	Ref.
D. Unclassified				
Boric Acid (10043-35-3)	Mouse (B6)	Hyperplasia	None	Dieter, 1994
Carbamazepine (298-46-4)	Rat (SD)	Adenoma	None	PDR, 1995r
Felbamate (25451-15-4)	Rat	Adenoma	Liver	PDR, 1995e
Flecainide (54143-55-4)	Rat (Wistar)	Adenoma	None	Case et al., 1984
Indomethacin (53-86-1)	Rat (SD)	Adenoma	None	Goerttler et al., 1992
Isopropanol (67-63-0)	Rat (F344)	Adenoma	None	Burleigh-Flayer et al., 1997
JP-4	Rat (F344)	Adenoma	Kidney	Bruner et al., 1993
d-Limonene (5989-27-5)	Rat (F344)	Adenoma	Kidney	Jameson, 1990
MTBE (1634-04-4)	Rat (CD, F344)	Adenoma	None	Belpoggi et al., 1995
Nicotine (54-11-5)	Rat (F344)	Hyperplasia (equivocal)	None	Thompson et al., 1973
Oxazepam (604-75-1)	Rat	Adenoma	Liver thyroid prostate	PDR, 1995n

*Strain is included when specified in citation.

Genotoxic Compounds that Produce LC Hyperplasia or Adenomas

Compound (CAS number)	Species (Strain)*	LC response	Other tumor sites	Ref.
1. Alkylating agents				
N-Nitrosobis-(2-oxopropyl)amine (BOP) (60599-38-4)	Rat (Wistar)	Adenoma	Prostate, vas deferens, coagulating glands, liver	Pour, 1986
1,3-Butadiene (106-99-0)	Rat (CD)	Adenoma	Pancreas	Owen et al., 1987
BrdU (59-14-3)	Rat (LIO)	Adenoma	Kidney, intestine	Anisimov, 1995
BrdU + X-rays (59-14-3)	Rat (LIO)	Adenoma	Prostate, kidney, adrenal cortex, hematopoietic system, thyroid	Anisimov and Osipova, 1993
3-Chloro-2-methylpropene (563-47-3)	Rat (F344)	Adenoma	Forestomach, kidney	NTP, 1986
Cycasin (14901-08-7)	Rat (ACI)	Adenoma	Intestine, liver, kidney	Fukunishi et al., 1971
Dibromochloropropane (DBCP) (96-12-8)	Humans	Hyperplasia	None	Cortes-Gallegos et al., 1980
Diethylnitrosoamine (DEN) (55-18-5)	Mice (RF)	Adenoma	Lung, liver, forestomach	Clapp, 1973
Dimethylnitrosoamine (DMN) (62-75-9)	Rat (Wistar)	Adenoma	Liver, hematopoietic system	Arai et al., 1979; Terao et al., 1978
Isoprene (78-79-5)	Rat (F344)	Adenoma	None	Melnick et al., 1994
8-Methoxypsoralen (298-81-7)	Rat (F344)	Adenoma	Kidney, Zymbal's, lung	Dunnick, 1989
Methy-CCNU (33073-59-5)	Mice (SJL/J)	Hyperplasia	None	Yegana et al., 1988
Nitrosoethylene (NEU) (759-73-9)	Rat (Outbred)	Adenoma	Nervous system	Ird and Smirnova, 1983
Streptozotocin (18883-66-4)	Rat (SD)	Adenoma	Pancreas, kidney, liver	Okawa and Doi, 1983
2. Base substitutions				
5-Azacytidine (320-67-2)	Rat (F344)	Adenoma	Hematopoietic system, skin, lung, kidney	Carr et al., 1988; Carr et al., 1984
Vidarabine (5536-17-4)	Rat	Adenoma	Liver, kidney, intestine, thyroid	Griffith, 1988
3. Metals				
Cadmium (7440-43-9)	Rat (Wistar/F344)	Adenoma	Lung, prostate, hematopoietic system	Bomhard et al., 1987; Waalkes and Rehm, 1992; Waalkes et al., 1997
4. Radiation				
X-Irradiation	Rat (Long-Evans)	Adenoma	None	Lindsay et al., 1969
5. Others				
Ethylene dichloride + Disulfiram (107-06-2/97-77-8)	Rat (SD)	Adenoma	Liver, skin	Cheever et al., 1990

* Strain is included when specified in citation.

Annex II

TABLE 3
Incidence of LCTs of the Testes in Male Rates for Studies Terminated at 24 Months

Strain	Diagnosis	Incidence	(%)	Ref.
Wistar	Adenoma	87/1249	7.0	Bomhard and Rinke, 1994
Crl:CD®BR	Adenoma	37/721	5.1	Hansen, 1993
Sprague-Dawley	Adenoma	16/349	4.6	Lewis, 1993
F344/DuCrj	Adenoma	537/569	94.4	Iwata et al., 1991
Crl:CD®BR	Adenoma	38/585	6.5	McMartin et al., 1992
Sprague-Dawley	Adenoma	11/1340	0.82	Chandra et al., 1992
	Carcinoma	1/1340	0.07	
Wistar	Adenoma	27/685	3.94	Walsh and Poteracki, 1994
F344	Adenoma	39,253/51,230	76.6	Mitsumori and Elwell, 1988
Crl:CD®BR	Adenoma	59/1260	4.68	Lang, 1992
	Carcinoma	1/1260	0.08	
CDF(F344)/CrlBR	Adenoma	741/946	78.3	Lang, 1990
Crl:CD®SD	Adenoma	29/496	5.8	IRI, 1995b

TABLE 4
Incidence of LCTs of the Testes in Male Mice for Studies Terminated at 24 Months

Strain	Diagnosis	Incidence	(%)	Ref.
Swiss CD-1®	Adenoma	—	≤1.0	Engelhardt et al., 1993
Crl:CD-1® BR	Adenoma	13/524	2.48	Lang, 1995
IRC Crj:CD-1®	Adenoma	8/891	0.9	Maita et al., 1988
CD-1®	Adenoma	14/725	1.9	Chandra and Frith, 1992
	Carcinoma	1/725	0.14	
B6C3F1	Adenoma	169/46,752	0.4	Mitsumori and Elwell, 1988
Crl:CD-1® Swiss	Adenoma	8/400	2.0	IRI, 1995a