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► **To cite this version:**

Thomas Nolte, Susanne Rittinghausen, Rupert Kellner, Eberhard Karbe, Birgit Kittel, et al.. RITA-Registry of Industrial Toxicology Animal data: The application of historical control data for Leydig cell tumors in rats. *Experimental and Toxicologic Pathology*, 2011, 63 (7-8), pp.645. 10.1016/j.etp.2010.05.006 . hal-00734584

HAL Id: hal-00734584

<https://hal.science/hal-00734584>

Submitted on 24 Sep 2012

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Accepted Manuscript

Title: RITA–Registry of Industrial Toxicology Animal data¹:
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PII: S0940-2993(10)00084-9
DOI: doi:10.1016/j.etp.2010.05.006
Reference: ETP 50492

To appear in:

Received date: 18-4-2010
Revised date: 9-5-2010
Accepted date: 11-5-2010

Please cite this article as: Nolte T, Rittinghausen S, Kellner R, Karbe E, Kittel B, Rinke M, Deschl U, RITA–Registry of Industrial Toxicology Animal data¹: The application of historical control data for Leydig cell tumors in rats, *Experimental and Toxicologic Pathology* (2008), doi:10.1016/j.etp.2010.05.006

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RITA – Registry of Industrial Toxicology Animal data *
The application of historical control data for Leydig cell tumors in rats

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Keywords: Leydig cell tumor, interstitial cell adenoma, Leydig cell adenoma, Leydig cell carcinoma, interstitial cell carcinoma, interstitial cell tumor, testis, testes, rat, rodent, carcinogenicity testing, carcinogenicity, oncogenicity, RITA, Registry of Industrial Toxicology Animal-data, historical control data

* RITA is funded jointly by *Abbott, AstraZeneca, BASF, Bayer, Boehringer Ingelheim, F. Hoffmann-La Roche, Merck-Serono, Novartis, Nycomed, Pfizer, Sanofi-Aventis, Schering-Plough, Syngenta*

Abstract

Historical data for Leydig cell tumors from untreated or vehicle treated rats from carcinogenicity studies collected in the RITA database are presented. Examples are given for analyses of these data for dependency on variables considered to be of possible influence on the spontaneous incidence of Leydig cell tumors.

In the 7453 male rats available for analysis, only one case of a Leydig cell carcinoma was identified. The incidence of Leydig cell adenomas differed markedly between strains. High incidences of close to 100% have been found in F344 rats, while the mean incidence was 4.2% in Sprague Dawley rats and 13.7% in Wistar rats. Incidences in Wistar rats were highly variable, primarily caused by different sources of animals. Mean incidences per breeder varied from 2.8% to 39.9%.

Analyses for the dependency on further parameters have been performed in Wistar rats. In breeders G and I, the Leydig cell tumor incidence decreased over the observation period and with increasing mean terminal body weight. The incidence of Leydig cell tumors increased with mean age at necropsy and was higher in studies with dietary admixture compared to gavage studies. These parameters had no effect on Leydig cell tumor incidence in breeders A and B. Animals from almost all breeders had a considerably higher mean age at necropsy when bearing a Leydig cell adenoma than animals without a Leydig cell adenoma. Studies with longitudinal trimming of the testes had a higher incidence than studies with transverse trimming.

The observed dependencies and breeder differences are discussed and explanations are given. Consequences for the use of historical control data are outlined. With the retrospective

analyses presented here we were able to confirm the published features of Leydig cell adenomas and carcinomas. This indicates that the RITA database is a valuable tool for analyses of tumors for their biological features. Furthermore, it demonstrates that the RITA database is highly beneficial for the definition of reliable historical control data for carcinogenicity studies on a scientifically solid basis.

Introduction

Leydig cell (LC) tumors, also called interstitial cell tumors, are the most frequent neoplasms in the testes of rats and mice. Their incidence varies according to strain, ranging from 1 – 5% in Sprague Dawley rats (Cook et al. 1999) to nearly 100% in F344 rats (e.g. Mitsumori and Elwell 1988). LC tumors occur less frequently in mice with an incidence of less than 1% to 2.5% (Cook et al. 1999).

LC tumors of rats are almost exclusively benign. Only isolated cases of LC carcinomas have been reported (e.g. Chandra et al. 1992, Lang et al. 1992).

The differential diagnosis between LC hyperplasia and adenoma is basically challenging, as proliferative LCs are usually cytologically not different from normal LCs except for large tumors (Ettlin et al. 1992). The differentiation between normal, hyperplastic and neoplastic LCs is, therefore, often arbitrary and generally based on the size of a lesion. According to the WHO/IARC and STP criteria, a LC mass is called hyperplasia if its diameter is equal to or less than 3 average seminiferous tubules in diameter and a LC adenoma if it is larger (Alison R et al. 1997, McConell et al. 1992).

LC hyperplasias and adenomas have been induced in rats by a large variety of chemical compounds and by different mode of actions (for review see Cook et al. 1999). Due to the arbitrary criteria for differentiation between LC hyperplasia and adenoma, the risk is high that a compound inducing exclusively LC hyperplasia may be falsely interpreted to induce also LC adenomas if tested in a strain with a low background incidence of LC adenomas (Davis TE 1995). Conversely, it is considered likely not to detect a tumorigenic effect on Leydig cells when F344 rats with their high background incidence are used. Historical control data for LC adenomas are, therefore, of high value to assist in the interpretation of rat carcinogenicity studies.

Three main databases exist for historical data on rodent carcinogenicity studies. These are the NTP database, the Charles River database and the RITA database. RITA, the Registry of Industrial Toxicology Animal-data, is a joint effort for the generation and maintenance of a database for rodent carcinogenicity and oncogenicity data from control animals. It has been funded in 1988 by 13 companies from the German and Swiss (agro-) chemical and pharmaceutical industry in co-operation with the Institute for Toxicology and Experimental Medicine (Fraunhofer ITEM) in Hannover, Germany, of the Fraunhofer-Gesellschaft for Applied Research. The project exists for more than 20 years now and is currently sponsored by 13 world-wide acting European and American companies.

The database structure and the related software programs have been set up by Fraunhofer ITEM. This includes a program for nomenclature, diagnostic criteria and images (goRENI), a program for data input (REGINA), and for data transfer from other pathology programs (RFI – Regina Foreign Input). The structure of the Oracle-based database has been described elsewhere in detail (Morawietz et al. 1992).

All histopathological diagnoses to be entered into the database are subject to a multi-step peer review process, also described by Morawietz et al. (1992). In brief, all proliferative lesions are reviewed by an experienced pathologist of the Fraunhofer Institute. Questionable, rare and interesting cases are brought forward to a panel of pathologists from RITA companies who decide by majority.

The RITA database does collect not only tumor data but also data on pre-neoplastic lesions. In addition, more than 50 single data items describing the experimental conditions are collected (Morawietz et al. 1992 and Table 1). These data items are compatible with the relevant variables identified by the STP working group on historical control data (Keenan et al. 2009). From its beginning the RITA database used a commonly agreed nomenclature and diagnostic criteria, later published by WHO/IARC.

In this article we present the historical background data for LC tumors in rats collected in the RITA database. We also give examples for analyses of these data on dependency of variables considered to be of possible influence on the spontaneous incidence of LC tumors.

Methods

Database

The WHO/IARC nomenclature and diagnostic criteria (Alison et al. 1997) were used for all Leydig cell tumors present in the database. The evaluations were performed on the database with status as of July 10, 2008, i.e. data from 134 rat studies (up to study no. 207) with 7453 male rats were analyzed. Data were extracted from the RITA database and evaluated by use of

common software programs (e.g. Microsoft EXCEL). Rats were purchased from the following suppliers: Charles River France, Charles River Germany, Charles River Italia, Charles River UK, Charles River USA, Ciba Geigy Ltd, Dr. Karl Thomae GmbH, Harlan-Winkelmann GmbH, Hoechst AG, RCC Ltd). Suppliers of rats were coded to ensure confidentiality of sponsors, as in the past some sponsors bred their own animals. Incidences were calculated on the number of organs examined, i.e. animals with testes missing or evaluation not possible were excluded.

Selection of variables

The historical data were analyzed against a number of variables considered to be of potential influence on the spontaneous incidence of LC adenomas. These variables were selected based on literature data (Table 2).

Generally, analyses were performed on a study basis. “Study” is used here and subsequently synonymous for the control group of a carcinogenicity or oncogenicity study. For the parameters “body weight” and “age at necropsy” also individual animal data were available. These data were analyzed for a difference between animals with LC adenomas and animals without LC adenomas.

For all other parameters, analyses were performed only if at least 10 studies were available. The investigations revealed strong strain and breeder differences. Therefore, dependencies on other variables were investigated for each strain and each breeder separately. In SD rats, the minimum number of studies (10) was available only for one breeder. A comparison of the studies for this breeder revealed considerable inhomogeneity with regard to the incidence of LC adenomas and the year of study start, body weight and mean age at necropsy. All these

variables were shown to have in Wistar rats potentially a considerable influence on the incidence of LC adenomas. Furthermore, 9 of the 10 studies were started between 1983 and 1988 and only one more recent study with start in 1995 was available. It was, therefore, decided not to perform further analyses for SD rats.

The variables investigated were checked for dependencies on each other. For this purpose, an isolated study with animals from breeder A which ran over a period of 31 months was excluded when investigating dependencies on mean age at necropsy.

Statistics

The number of animals per study was considered when calculating the mean incidence of LC adenomas among multiple studies (weighed means). Correlation coefficients and their statistical significance were calculated by use of Statistica version 8.0 where appropriate. A correlation was considered weak when the absolute value of the correlation coefficient was ≥ 0.3 and < 0.5 , moderate when it was ≥ 0.5 and > 0.7 and strong when it was ≥ 0.7 .

Results

LC carcinomas

There was only one Leydig cell carcinoma present in the database (134 studies and 7453 male rats) which occurred in a 24-month old Crl:WI (Han) rat (Groeters et al. 2009). It was an unusual case as no solid and circumscribed tumor mass could be identified in the testes. The Leydig cell proliferation rather appeared as a diffuse hyperplasia with atypia. However, one grossly enlarged iliac lymph node contained a large accumulation of cells with Leydig cell

morphology. After immunohistochemical proof of their Leydig cell nature by showing a positive reaction for inhibin α , calretinin, S-100, and vimentin, this mass was interpreted to be a metastasis of a primary Leydig cell carcinoma. Consequently, the RITA panel diagnosed a Leydig cell carcinoma in one testis.

LC adenomas

The incidence of LC adenomas showed a strong **strain** dependency (Figure 1). The two RITA studies conducted in F344 rats had a high incidence of 76% and 90%, respectively. SD rats had the lowest incidence with a mean of 4.2%, a minimum of 0% and a maximum of 10%. The incidence was highly variable in Wistar rats with a minimum of 0%, a maximum of 60% and a mean of 13.7%. Due to these marked strain differences, further investigations were performed separately for Wistar and SD rats.

In Wistar rats strong differences between **breeders** were evident. The lowest mean incidence was 2.8% for breeder B, the highest 39.9% for breeder A (Figure 2, Table 3). Due to these marked differences, further analyses in Wistar rats have been performed for animals from each breeder separately. The breeder influence was less pronounced in SD rats where the lowest mean incidence was 2.3% for breeder D3 and the highest 6.3% for breeder H. Means and variability were similar for breeders D1, D2 and H (Figure 3).

The various sources of Wistar rats showed differences in their dependency on the **year of study start** (Figures 4 - 7, Table 4). There was no correlation of both variables for breeders A, B and G. A statistically significant negative correlation was evident for breeder I. The correlation was highly significant, when four studies with an extraordinary high incidence were excluded. The data available for breeder D1 did not allow a valid analysis due to the

marginal number of studies and an extraordinary high incidence for one study. No further analyses were performed with data from breeder D1 due to this data constellation.

Wistar rats from breeder A and B showed no correlation between the incidence of LC adenomas and group mean **terminal body weight** (Figures 8 +9, Table 4). In Wistar rats from breeders G and I, the incidence of LC adenomas decreased with increasing group mean terminal body weight (Figures 10 + 11). The moderate negative correlation of both parameters was statistically significant (Table 4). The correlation was less marked in breeder I, when the studies with extraordinary high values were excluded.

An additional analysis was performed for a possible difference in the mean terminal body weight between animals with LC adenoma when compared to animals without LC adenoma. Again, this analysis was performed for each strain separately. Generally, only minor differences were found which were inconsistent between strains and breeders and ranged between an increase by 1.9% to a decrease by 2.7% (Table 5). An increase by 10.9% was observed in F344 rats with LC adenoma when compared to those without LC adenoma. This high difference is interpreted as a chance observation, resulting from the low number of animals without LC adenomas (N = 17).

The analysis for dependency on the group mean **age at necropsy** revealed no correlation for breeders A and B but a significant positive correlation for breeders G and I (Figures 12 - 15, Table 4). When analyzed on an animal basis, a clear difference in the mean age at necropsy was evident for animals with LC adenomas when compared to animals without LC adenomas for all breeders. Generally, tumor bearers lived 38 days (breeder A) to 59 days (breeder I) longer than animals without LC adenomas (Table 6). This difference in mean longevity was also found for SD rats. F344 rats with LC adenoma lived in average 224 days longer than

those without LC adenoma. Again, the low number of animals without LC adenomas (N = 17) has to be considered when interpreting this finding.

Differences dependent on the **route of administration** were evident for dietary admixture vs. oral gavage in Wistar rats from breeders G and I only (Figures 16 and 17, Table 7). Mean as well as minimum and maximum incidences were higher in studies with dietary admixture. A high incidence of LC adenomas was noted in each two studies of animals from breeder G + I, in which animals were treated by subcutaneous injection. Subcutaneous injection was the route of administration in only these four studies conducted in Wistar rats. The incidence of LC adenomas in these studies, however, was well within the range of studies conducted by dietary administration.

An analysis for dependency on **individual caging vs. group caging** was not possible as studies with animals from a given breeder were usually performed either by single housing or by group housing.

Only animals from breeders A and I could be discriminated for the **orientation of trimming** the testes (Table 8). For breeder A, the incidence of LC adenomas was only slightly higher when the testes were trimmed longitudinally compared to transverse trimming. A difference related to the orientation of trimming was more obvious for animals from breeder I, where longitudinal trimming resulted in an incidence of 14% compared to 7.9% after transverse trimming.

An analysis for the influence of the **vehicle** was not possible due to the low number of studies per vehicle. "Vehicle" is a facultative variable in the database and information on the vehicle

used is given for less than 50% of the studies. Furthermore, 9 different vehicles have been used in studies with oral administration in Wistar rats. This resulted in a maximum number of studies per vehicle of 7, followed by 4.

The analysis for **dependencies between the variables** investigated revealed prominent differences between gavage studies and studies with dietary admixture with regard to mean body weight and study start. Studies with dietary admixture were in average conducted 6 and 13 years earlier with animals from breeders G and I, respectively. The terminal mean body weight of animals treated with dietary admixture was 19% lower for breeder G and 8% lower for breeder I (Table 9).

The mean terminal body weight of Wistar rats showed for all breeders a moderate to strong negative correlation to the mean age at necropsy (Figures 18 - 22, Table 10). The mean terminal body weight increased in breeders G and I over the observation period (Figures 23 - 27, Table 10). For breeder A the increase was statistically significant only, when an extreme value was excluded. No correlation of both parameters could be established in case of breeders B and D1.

There was no statistically significant dependency between mean age at necropsy and date of study start for breeders A, B, D1, and G. In contrast, both parameters showed a highly significant negative correlation for breeder I (Figures 28 – 32, Table 10).

Discussion

LC carcinomas:

Only one case of a LC carcinoma was available in the RITA database (7453 male rats). This isolated occurrence of LC carcinomas is in line with the few published cases: Mean incidences between 0.07% and 0.2% with a maximum incidence of 3.3% were reported in SD rats (Mc Martin et al. 1992, Chandra et al. 1992, Lang 1992, Giknis and Clifford 2004). No LC carcinoma was found in 465 Wistar Crl(WI)BR rats (Potracki and Walsh 1998), while one out of 10 studies conducted with Wistar Han rats had two LC carcinomas, resulting in an incidence of 3.64% (Giknis and Clifford 2003).

LC adenomas:

Strain and breeder: The high incidence in the two studies with F344 rats of 76% and 90% is in line with the studies in the NTP database which usually have an incidence of LC adenomas > 90%. Published incidences are in a comparable range: Iwata et al. (1991) found LC adenomas in 94.4% of F344DuCrj rats, Lang (1990) in 78.3% of CDF(F344)CrlBR rats, and Mitsumori and Elwell (1988) in 76.6% of not further specified F344 rats.

The RITA data for LC adenomas in SD rats from different breeders (means between 2.3% and 6.3%) are comparable to published data indicating a spontaneous incidence between 1 and 5% (e.g. Cook et al. 1999). The mean for LC adenomas in the Charles River compilation, comprising 2146 male SD rats, is with 2.4% also in this range (Giknis and Clifford 2004). A considerably lower mean incidence of 0.8% was reported in 1340 SD rats (Chandra et al. 1992). The maximum incidence of 10% found in a RITA study was comparable to the maximum incidence of 9.33% in the Charles River compilation.

Variations within strains depending on breeder have been described (van Zwieten et al. 1988, Deschl et al. 2002) and became particularly obvious in Wistar rats in our investigations. The mean incidence was with 39.9% by far higher in breeder A than in the other breeders, where it ranged between 2.8% and 12.5%. Published background incidences in Wistar rats are in the range of the latter breeders (e.g. Bomhard and Rinke 1994, Walsh and Poteracki 1994), confirming the outstanding high spontaneous incidence in breeder A.

Strong breeder dependent differences in the spontaneous incidence of a given tumor as described here for the LC adenoma are reflected in a recommendation of the USEPA (2005) stating that the most relevant HCD generally come from the same animal supplier.

Year of study start: The background for the analysis on a dependency between LC adenoma incidence and year of study start was the general presumption that genetic drift may occur over time which may result in changes in the reported frequency of certain pathologic lesions. The present analysis revealed no dependency between year of study start and incidence of LC adenomas for some breeders indicating stability of the background incidence over time and, therefore, no need to apply a time window when defining the reliability of historical control data. A negative trend was evident for other breeders indicating the necessity of a time window. The definition of a time window is to some extent arbitrary but should result in considerable stability. Breeder I may serve as an example: There was a statistically significant negative correlation between year of study start and incidence of LC adenomas when all studies were included. Exclusion of studies started between 1983 and 1993 would result in considerable stability in the incidence of the remaining 14 studies and no correlation between both variables.

The relevant guidelines address the topic of genetic drift directly or indirectly and define a time window of 2 – 3 years prior to or following the study (USEPA 2005), 5 years prior to the study in question (EMEA 2002), 5 years prior to and 2 – 3 years after study conduct (OECD 2002). However, the results presented here confirm the conclusion of Deschl et al. (2002) that some parameters may change in a short period of time while others remain stable over prolonged periods. We conclude that a time window should be defined for each lesion after analysis of the dependency between incidence and year of study start. The use of a “fixed moving time window” may lead to loss of important information or the reference to inappropriate historical control data.

We consider that the reason for the decrease in the incidence of LC adenomas during the observation period in animals from breeder I is the strong concomitant increase in mean terminal body weight. The causal relationship between an increase of body weight and a reduction of LC adenoma incidence is described in the following chapter.

Dependency on body weight: It is well documented, that the incidence of some endocrine tumors increases with body weight and that this is associated with increased serum prolactin levels (Roe et al. 1995). However, there is strong evidence that increased body weight and associated increases in prolactin serum levels reduce the LC tumor incidence in rats: It has been demonstrated, that hyperprolactinemia reduced pituitary LH and FSH secretion (Shape and McNeilly 1979), leading to a reduced stimulation of LCs. Conversely, dopamine agonists, which reduce serum prolactin levels, have been shown to cause LC adenomas in rats (Prentice et al. 1992, Roberts et al. 1993, Yamada et al. 1994), mediated by increased LH secretion. As expected, the incidence of LC adenomas decreased markedly with increasing body weight in breeders G and I.

There was no obvious difference in the mean terminal body weight between animals with LC adenomas and animals without LC adenomas. This does not support a previous observation that rats with LC tumors developed food aversion, anorexia, and hypophagia (Bernstein and Fenner 1983; Bernstein et al. 1985).

The negative correlation between body weight and LC adenoma incidence is considered to be the underlying reason for the reduction of LC adenoma incidence during the observation period in breeder I: In male rats from this breeder, the mean terminal body weight increased considerably during the 18-year observation period.

Dependency on mean age at necropsy: LC tumors generally occur in older animals (Cook et al. 1999). In F344 rats their occurrence before 1 year age is rare but rises rapidly before and after 2 years of age (Turek and Desjardins 1979, Boorman et al. 1990). However, only breeders G and I showed the expected positive correlation between incidence of LC adenomas and mean age at necropsy, while there was no correlation between both parameters for breeders A and B. When analyzed on an animal basis it became obvious that animals with LC adenomas lived longer than animals without LC adenomas. This seems to be unexpected but is considered to be caused by the interplay of (I) the increased likelihood to develop a LC adenoma with increasing age and (II) by the fact that LC adenomas are generally not fatal and, therefore, their presence does not reduce the survival.

Dependency on route of administration: The analysis for dependency on the route of administration revealed a higher incidence in studies with dietary admixture compared to studies with gavage administration for breeders G and I. However, studies with dietary admixture usually started earlier than studies with gavage administration with a difference of 6 years for breeder G and 13 years for breeder I. This is considered important as studies with

animals from both breeders showed a negative correlation between year of study start and incidence of LC adenomas. Also of relevance is in this context the lower mean body weight in animals treated by dietary admixture, as animals from both breeders showed a negative correlation between mean body weight and incidence of LC adenomas. Both dependencies are considered to explain the observed difference in the incidence of LC adenomas between studies with dietary admixture and gavage administration.

The subcutaneous route of administration was used in four studies conducted with Wistar rats. Two of these studies used animals from breeder I, and both had a high incidence of LC adenomas. We interpret this as a chance observation unrelated to the route of administration as (I) comparably high incidences occurred in other studies with animals from breeder I and (II) the incidence of LC adenomas in the two other studies with subcutaneous injection was well within the range of studies conducted by dietary administration with animals from the same source.

Orientation of trimming: As expected, longitudinal trimming resulted in a higher incidence of LC adenomas due to the larger tissue surface available for examination. This result confirmed the recommendation for longitudinal trimming made by the Society of Toxicologic Pathology (STP, McConnell et al. 1992). Also the Registry of Industrial Toxicology Animal Data (RITA) considers longitudinal trimming as an option, second to its recommendation of transverse trimming (Kittel et al. 2004).

Individual caging vs. group caging: Nyska and co-workers (1998) observed an inverse relation of pituitary gland pars distalis adenomas and testicular Leydig cell tumors in NTP inhalation and dermal studies in F344 rats. When compared to gavage and feeding studies, the incidence of pars distalis adenomas was increased, that of LC adenomas decreased. Animals

were individually caged in dermal and inhalation studies but group caged in gavage and feeding studies. Again, the authors explained this difference by a potentially higher stress level of individually housed male rats compared to group housed animals. The distribution of data in the RITA database did not allow an analysis on the influence of this parameter. Studies performed with animals from a given breeder were usually performed either by individual caging or by group caging.

Vehicle: The type of vehicle may have a major influence on body weight and body weight development, based on its caloric density. A vehicle may considerably add to the daily caloric intake and, therefore, vehicle related effects on tumor incidences would not be unexpected and have been described (e.g. Rao et al. 1987). However, vehicle effects seem to be multifactorial as in the study from Rao et al. (1987) the use of corn oil was associated with a higher survival rate in F344 rats, due to a lower incidence of fatal lymphomas, but without an effect on the incidence of LC adenomas. A valid analysis of the RITA data for a vehicle effect could not be performed due to the numerous different vehicles used and the low number of studies per vehicle.

Dependencies between variables investigated: There was an obvious increase in mean terminal body weight over the observation periods for all breeders. This is a well-known trend and has been described by many authors (e.g. Rao et al. 1990). This trend is considered to be the main factor responsible for the reduction of LC adenoma incidences during the observation period in animals from breeders G and I. Animals from those breeders showed also the most obvious increase of mean terminal body weight.

The increase in mean terminal body weight in animals from certain breeders was associated with a slight reduction in mean age at necropsy, indicating a slight reduction of longevity.

However, the effect was minimal and the mean age at necropsy was in most studies well above 700 days indicating a high survival until study termination.

The negative correlation between mean terminal body weight and mean age at necropsy may be unexpected considering that the body weight of rats generally increases with age.

However, it does plateau or may even slightly decrease towards the end of the animals' natural lifespan. The lower mean body weight in studies with higher mean age at necropsy does much more reflect the well established dependency between body weight and longevity: food restriction and the resulting lower body weight leads to an increase longevity when compared to ad libitum fed animals (e.g. Roe et al. 1995).

The analyses presented here show that the value of a historical database for tumor incidence from carcinogenicity studies is dependent on both the number of studies and the variables collected. Valuable analyses can be performed only if a sufficient number of similar studies are available. As the animal strain proved to be the most relevant factor, it would be advantageous if sponsors would focus on one strain. In this respect it is encouraging that the Wistar rat seems to become the favorite strain for safety testing in chemical and pharmaceutical industry.

With a retrospective analysis of the RITA database for LC tumors we were able to confirm the published features of LC adenomas and carcinomas. This indicates that the RITA database is a valuable tool for analyses of the biological features of tumors from rodent carcinogenicity studies. Furthermore, it demonstrates that the RITA database is highly valuable for the definition of reliable historical control data for carcinogenicity studies on a scientifically solid basis.

Acknowledgement:

The authors are grateful to all the more than one hundred pathologists who worked actively on the RITA project over the past 20 years. They wish to particularly acknowledge the extraordinary engagement of Gerd Morawietz in the design and continuation of the database. Carina Ittrich and Volker Krzykalla are acknowledged for their assistance in statistical aspects of data interpretation.

References:

- Alison R, Ettlin RA, Foley GI, Harleman JH, Maekawa A, McConnell RF, Quereshi SR, Rehm S, Reznik G, Valerio M. 8. Male genital system. *In*: Mohr U, Capen CC, Dungworth DL, Griesemer RA, Ito N, and Turusov US (eds.), *International Classification of Rodent Tumours. Part I: The Rat*, IARC Scientific Publication No. 122, Lyon, France: IARC (WHO), 1997.
- Bernstein IL, Fenner DP. Learned food aversions: heterogeneity of the animal models of tumor induced anorexia. *Appetite* 1983;4:79-86.
- Bernstein IL, Treneer CM, Kott JH. Area postrema mediates tumor effects on food intake, body weight and learned aversions. *Am J Physiol* 1985;249:296-300.
- Bomhard E, Rinke M. Frequency of spontaneous tumors in Wistar rats in 2-year carcinogenicity studies. *Exp Toxicol Pathol* 1994;46:17-29.
- Boorman GA, Eustis SL, Elwell MR. Neoplasms of the testis. *In*: Stinson SF, Schuller HM, Reznik G (editors): *Atlas of tumor pathology of the Fischer rat*. Boca Raton: CRC Press 1990. pp 409-414.
- Chandra M, Riley MGI, Johnson DE. Spontaneous neoplasms in aged Sprague Dawley rats. *Arch Toxicol* 1992;66:496-502.
- Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM, Foster PMD. Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. *Crit Rev Toxicol* 1999;29(2):169-261.
- Davis TE. Interstitial cell adenoma of the rat testis: why is it such a problem in toxicology studies using Sprague Dawley rats? *Toxicol Pathol* 1995;23(1):83-86.
- Deschl U, Kittel B, Rittinghausen S, Morawietz G, Kohler M. The value of historical control data – scientific advantages. *Toxicol Pathol* 2002;30(1):80-87.
- EMA CPMP Note for guidance on carcinogenic potential. EMA CPMP/SWP/2877/00, London, 25 July 2002. Available on web site <http://www.emea.europa.eu/pdfs/human/swp/287700en.pdf>.
- Ettlin RA, Quereshi SR, Perentes E, Christen H, Gschwind R, Buser MW, Oberholzer M. Morphological, immunohistochemical, stereological and nuclear shape characteristics of proliferative Leydig cell alterations in rats. *Path Res Pract* 1992;185:643-648.
- Giknis MLA, Clifford CB. Compilation of spontaneous neoplastic lesions and survival in Crl:CD[®](SD) rats from control groups. Charles River (2004) on request.
- Giknis MLA, Clifford CB. Spontaneous neoplasms and survival in Wistar Han rats: Compilation of control group data. Charles River (2003) on request.
- Groeters S, Kaufmann W, Nolte T (2009): 6th European Congress of Toxicologic Pathology Presentation Abstracts of the Joint ETS/ESTP Congress, September 24–26, 2008, Edinburgh, Scotland. Slide Seminar Case Presentation 6: Carcinoma interstitial cell (Leydig cell). *Exp Toxicol Pathol* 61:401-402.

Haseman JK. Body weight-tumor incidence correlations in long-term rodent carcinogenicity studies. *Toxicol Pathol* 1997;25(3):256-263.

Keenan C, Elmore S, Francke-Carroll S, Kemp R, Kerlin R, Peddada S, Pletcher J, Rinke M, Schmidt SP, Taylor I, Wolf DC. Best Practices for use of historical control data of proliferative rodent lesions. *Toxicol Pathol* 2009;37: 679 - 693.

Iwata H, Hirouchi Y, Koike Y, Yamakawa S, Kobayashi K, Yamamoto T, Inoue H, Enomoto M. Historical control data on non-neoplastic and neoplastic lesions in F344/DuCrj rats. *J Toxicol Pathol* 1991;4:1-24.

Lang PL (1990) Spontaneous neoplastic lesions in the CDF(F344)CrI Br rat, Charles River Laboratory, Raleigh.

Lang PL (1992) Spontaneous neoplastic lesions and selected non-neoplastic lesions in the CrI:CD Br rat, Charles River Laboratory, Raleigh.

McConnell RF, Westen HH, Ulland BM, Bosland MC, Ward JM. Proliferative lesions of the testes in rats with selected examples from mice. In: *Guides for Toxicologic Pathology*. Washington DC: STP/ARP/AFIP; 1992.

Mc Martin DN, Sahota PS, Gunson DE, Hsu HH, Spaet RH. Neoplasms and related proliferative lesions in control Sprague Dawley rats from carcinogenicity studies. Historical data and diagnostic considerations. *Toxicol Pathol* 1992;20:212-225.

Mitsumori K, Elwell MR. Proliferative lesions in the male reproductive system of F344 rats and B6C3F1 mice: incidence and classification. *Environ Health Perspect* 1988;77:11-21.

Morawietz G, Rittinghausen S, Mohr U. RITA – Registry of Industrial Toxicology Animal Data – Progress of the working group. *Exp Toxicol Pathol* 1992;44:301-309.

Nyska A, Leininger JR, Maronpot RR, Haseman JK, Hailey JR. Effect of individual versus group caging on the incidence of pituitary and Leydig cell tumors in F344 rats: propose mechanism. *Medical hypotheses* 1998;50(6):525-529.

OECD Environment, Health and Safety Publications. Series on testing and assessment no. 35 and Series on Pesticides No. 14. Guidance Notes for analysis and evaluation of chronic toxicity and carcinogenicity studies. ENV/JM/MONO 2002;19. Available at Web site [http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono\(2002\)19](http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)19) .

Poteracki J, Walsh KM Spontaneous neoplasms in control Wistar rats: a comparison of reviews. *Toxicol Sci* 1998;45:1-6.

Prentice DE, Siegel RA, Donatsch P, Quereshi S, Ettlin RA. Mesengerline induced Leydig cell tumours, a syndrome involving the pituitary-testicular axis of the rat. *Arch Toxicol* 1992;15(Suppl.):197-204.

Prentice RL, Smythe RT, Krewski D, Mason M. On the use of historical control data to estimate dose response trends in quantal bioassays. *Biometrics* 1992;48(2):459-478.

Nolte et al: RITA - The application of historical control data for Leydig cell tumors in rats

Rao GN, Haseman JK, Grumbein S, Crawford DD, Eustis SL. Growth, body weight, survival and tumor trends in F344/N rats during an eleven-year period. *Toxicol Pathol* 1990;18(1):61-70.

Rao GN, Piegorsch WW, Haseman JK. Influence of body weight on the incidence of spontaneous tumors in rats and mice of long-term studies. *Am J Clin Nutr* 1987; 45:252-260.

Roberts SA, Robinson RL, VanRyzin RJ, Stoll RE. Increased incidence of Leydig cell tumours in the rat with the dopaminergic agonist, SDZ 205-502: Correlation with increased serum LH levels. *Toxicologist Abstr.* 1993;13.

Roe FJC, Lee PN, Conybeare G, Kelly D, Matter B, Prentice D, Tobin G. The biosure study: Influence of composition of diet and food consumption on longevity, degenerative diseases and neoplasia in Wistar rats studied for up to 30 months post weaning. *Food and Chem Toxicol* 1995;33(Supplement 1):S1-S100.

Kittel B, Ruehl-Fehlert C, Morawietz G, Klapwijk J, Elwell MR, Lenz B, O'Sullivan MG, Roth DR, Wadsworth PF. Revised guides for organ sampling and trimming in rats and mice – Part 2: A joint publication of the RITA and NACAD groups. *Exp Toxicol Pathol* 55(6):413-431.

Shape RM, McNeilly AS. The effect of induced hyperprolactinemia on Leydig cell function and LH-induced loss of LH-receptors in the rat testis. *Mol Cell Endocrinol* 1979;16(1):19-27.

Turek FW, Desjardins C. Development of Leydig cell tumors and onset of changes in the reproductive and endocrine systems of aging F344 rats *J Natl Cancer Inst* 1979;6:969-975.

USEPA: Guidelines for carcinogen risk assessment. EPA/630/P-03/001B. Available at Web site <http://www.epa.gov/iris/backgr-d.htm> (2005).

Van Zwieten MJ, Majka JA, Peter CP, Burek JD. The value of Historical Control Data. In: Grice HC, Ciminera JL (eds) *ILSI Monographs: Carcinogenicity — The design, analysis and interpretation of long-term animal studies*. Berlin: Springer-Verlag, 1988; pp 39–51.

Walsh KM, Poteracki J. Spontaneous neoplasms in the control Wistar rat. *Fundam Appl Toxicol* 1994;22:65-72.

Yamada T, Maita K, Nakamura J, Murakami M, Okuno Y, Hosokawa S, Matsuo M, Yamada H. Carcinogenicity studies of oxolinic acid in rats and mice. *Food Chem Toxic* 1994;32:397-408.

Tables:

Table 1: Categories of variables collected in the RITA database and examples thereof.

Category	Number of variables	Examples
Study	17	<ul style="list-style-type: none"> • Species • Strain • Animal supplier • Laboratory which conducted the study • Type of study (carcinogenicity or chronic toxicity/oncogenicity) • Duration of study (males/females)
Animal housing	22	<ul style="list-style-type: none"> • SPF conditions • Number of animals per cage (males/females) • Males and females in the same / separate rooms • Mean light intensity • Type of bedding
Feeding	10	<ul style="list-style-type: none"> • Ad libitum / restricted • Diet type • Diet constitution • Diet consumption at a given day (group average)
Treatment	7	<ul style="list-style-type: none"> • Treatment yes / no • Route of administration • Type of vehicle
Serial observations	1	<ul style="list-style-type: none"> • Clinical observation frequency
Protocol organs	4 per organ	<ul style="list-style-type: none"> • Number of embedded tissues / samples • Plane of section • Examination of paired organs • Trimming according to guideline
Animal	10	<ul style="list-style-type: none"> • Days under test • Age at death • Death status • Cause of death • Body weight at specific dates
Histological findings	Up to 9	<ul style="list-style-type: none"> • Multiplicity • Bilateral occurrence • Malignancy • Metastasis • Invasion • Modifier / qualifier

Table 2: Variables for which an influence on the spontaneous incidence on LC adenomas is documented in literature or for which an influence is considered likely.

Variable	Influence on incidence of LC adenomas	Reference
Strain	SD < Wistar < F344	Cook et al. 1999 Mitsumori and Elwell 1988
Breeder	Variation between breeders	van Zwieten et al. 1988 Deschl et al. 2002
Year of study start	Effect of genetic drift on any tumor type	e.g. OECD 2002, EMEA 2002, USEPA 2005
Route of administration	Lower incidence in dermal and inhalation studies	Nyska et al. 1998
Group mean terminal body weight	Increased incidence of endocrine tumors with increased body weight	e.g. Roe et al. 1995
Group mean age at necropsy	Increased incidence with mean age	Cook et al. 1999
Individual caging vs. group caging	Reduced incidence	Haseman et al. 1997
Orientation of tissue trimming	Increase of surface area examined increases the likelihood to detect a tumor	e.g. McConnell et al. 1992
Vehicle	Various effects, e.g. on body weight	Rao et al. 1987

Table 3: Strain- and breeder dependent differences in the incidence of LC adenomas in rats.

Strain	Breeder	N studies	N animals	Incidence [%]		
				Mean	Min.	Max.
Wistar	A	19	1079	39.9	18	60
	B	12	800	2.8	0	6.0
	D1	11	608	5.8	0	29.0
	G	16	770	12.5	4.0	22.0
	I	30	1609	6.8	1.1	22.0
SD	D1	10	518	5.4	0	12
	D2	5	279	5.0	1.7	10
	D3	8	440	2.3	1.8	3.6
	H	6	398	6.3	2.0	8.6
F344	D6	2	100	83.0	76.0	90.0

Table 4: Incidence of Leydig cell adenomas in Wistar rats from different breeders – correlation to study start, group mean body weight and group mean age at necropsy.

	N studies	Correlation (r- and p-values)					
		Study start		Body weight		Age at necropsy	
		r	p	r	p	r	p
Breeder A	19	-0.119	0.627	-0.014	0.953	0.035	0.887
Breeder B	12	-0.108	0.738	-0.159	0.623	-0.219	0.469
Breeder G	16	-0.338	0.201	-0.516	0.041	0.512	0.043
Breeder I	26	-0.438	0.015	-0.544	0.002	0.468	0.009
Breeder I *	22	-0.513	0.007	-0.650	< 0.001	0.604	0.001

* studies with extraordinary high values excluded

Table 5: Mean terminal body weight: breeder-related comparison of animals with LC adenomas with animals without LC adenomas.

	N animals with LC adenoma	Mean terminal body weight [g]		
		With LC adenoma	Without LC adenoma	Δ %
Wistar Breeder A	430	663	659	0.6
Wistar Breeder B	22	543	541	0.4
Wistar Breeder D1	35	514	525	-2.1
Wistar Breeder G	96	482	495	-2.6
Wistar Breeder I	109	465	478	-2.7
F344	83	326	294*	10.9
SD	86	683	681	0.3

* N = 17

Table 6: Mean age at necropsy: breeder-related comparison of animals with LC adenomas with animals without LC adenomas

	N animals with LC adenoma	Mean age at necropsy [days]		
		With LC adenoma	Without LC adenoma	Δ [days]
Wistar Breeder A	430	782	744	38
Wistar Breeder B	22	803	777	26
Wistar Breeder D1	35	774	739	35
Wistar Breeder G	96	884	825	59
Wistar Breeder I	109	787	747	40
F344	83	762	538*	224
SD	86	748	717	31

* N = 17

Table 7: Incidence [%] of LC adenomas in Wistar rats dependent on route of administration and separated for breeders.

		Route of administration					
		No application	Feeding	Oral gavage	Drinking water	Inhalation	s.c. injection
Breeder A	N =	5	12	1	0	1	0
	Mean	41.7	38.0	46.0	-	42.0	-
	Min.	30.0	18.0	-	-	-	-
	Max.	52.5	60.0	-	-	-	-
Breeder B	N =	6	6	0	0	0	0
	Mean	3.0	2.5	-	-	-	-
	Min.	0.0	2.0	-	-	-	-
	Max.	6.0	4.0	-	-	-	-
Breeder D1	N =	3	2	5	0	1	0
	Mean	2.0	5.9	3.3	-	29.6	-
	Min.	0.0	3.8	2.0	-	-	-
	Max.	4.0	7.8	6.0	-	-	-
Breeder G	N =	0	9	5	0	0	2
	Mean	-	15.2	6.8	-	-	15.0
	Min.	-	5.0	4.0	-	-	12.0
	Max.	-	22.0	10.0	-	-	18.0
Breeder I	N =	2	19	6	1	0	2
	Mean	4.0	7.4	2.6	6.0	-	20.0
	Min.	4.0	1.7	1.1	-	-	18.0
	Max.	4.0	22.0	6.1	-	-	22.0

Table 8: Differences in the incidence of LC adenomas in Wistar rats from breeders A and I in dependence on the orientation of trimming.

	Trimmed longitudinally		Trimmed transversely	
	N all animals	% animals with LC adenoma	N all animals	% animals with LC adenoma
Breeder A	399	47.9	90	40.0
Breeder I	100	14.0	809	7.9

Table 9: Analysis of studies with animals from breeder G and I separately for dietary admixture and oral gavage.

Breeder	Route of administration	Mean incidence of LC adenomas	Mean body weight	Mean study start
G	Dietary admix.	15.2	478	July 1988
	Oral gavage	6.8	569	April 1994
I	Dietary admix.	7.4	471	March 1984
	Oral gavage	2.6	507	November 1997

Table 10: Relations between variables investigated in Wistar rats, separated by breeders: Correlation coefficients r and related p-values.

	Body weight – dependency on				Age at necropsy – dependency on	
	Age at necropsy		Date study start		Date study start	
	r	p	r	p	r	p
Breeder A(*)	-0.493	0.032	0.195 (0.552)	0.424 (0.018)	-0.273 (0.08)	0.257 (0.740)
Breeder B	-0.652	0.021	0.317	0.315	0.038	0.284
Breeder D1	-0.854	<0.001	0.339	0.307	-0.355	0.284
Breeder G	-0.611	0.012	0.798	<0.001	-0.262	0.327
Breeder I	-0.524	0.003	0.758	<0.001	-0.547	0.002

* in parentheses: extreme value excluded

Figures:

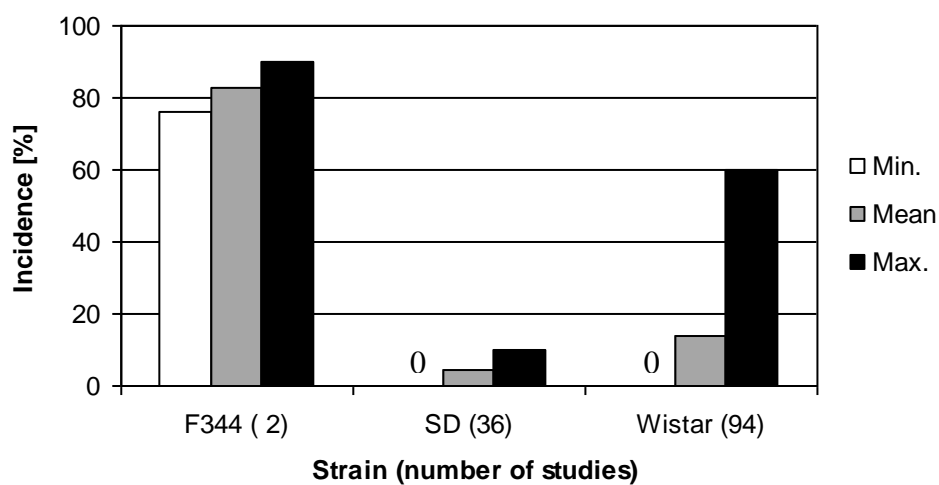


Figure. 1: Incidence of LC adenomas in rats, strain differences.

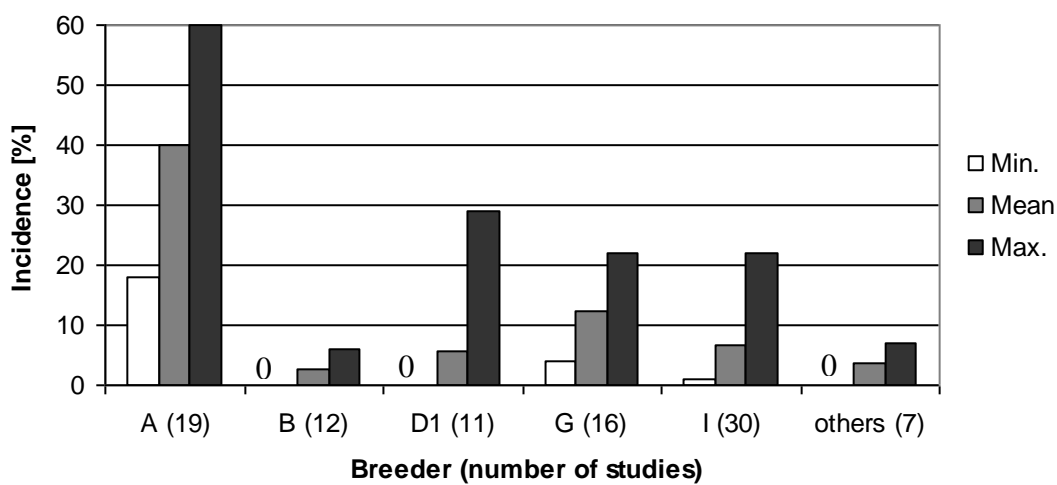


Figure 2: Incidence of LC adenomas in Wistar rats, breeder differences.

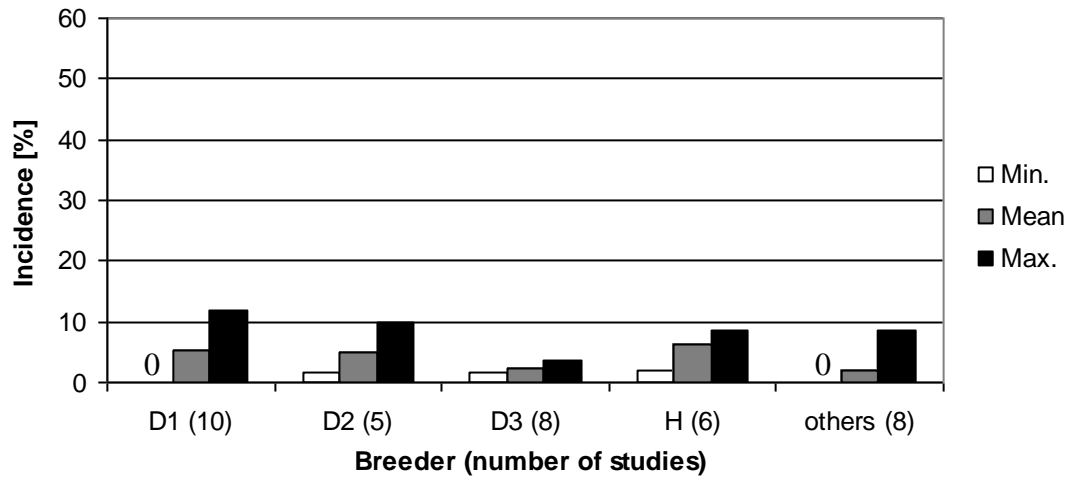


Figure 3: Incidence of LC adenomas in Sprague Dawley rats, breeder differences.

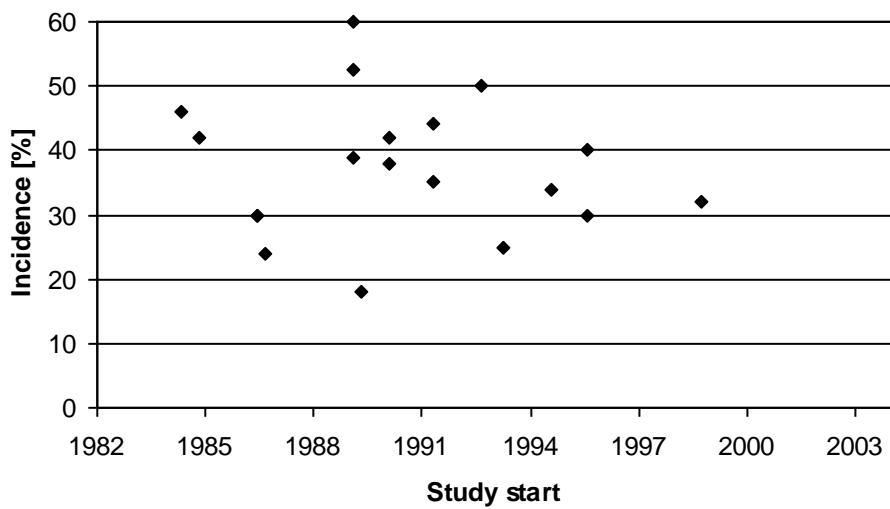


Figure 4: Incidence of LC adenomas in Wistar rats from breeder A: no dependency on year of study start.

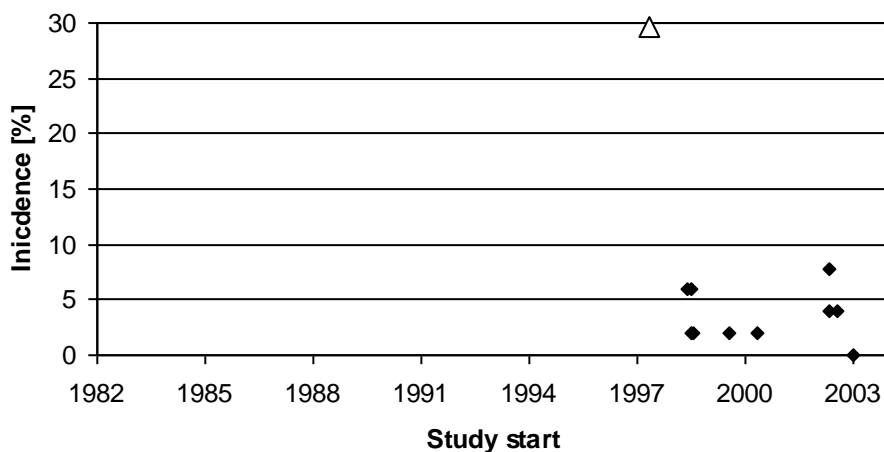


Figure 5: Incidence of LC adenomas in Wistar rats from breeder D1: limited data set with an extreme value (triangle), no analysis performed.

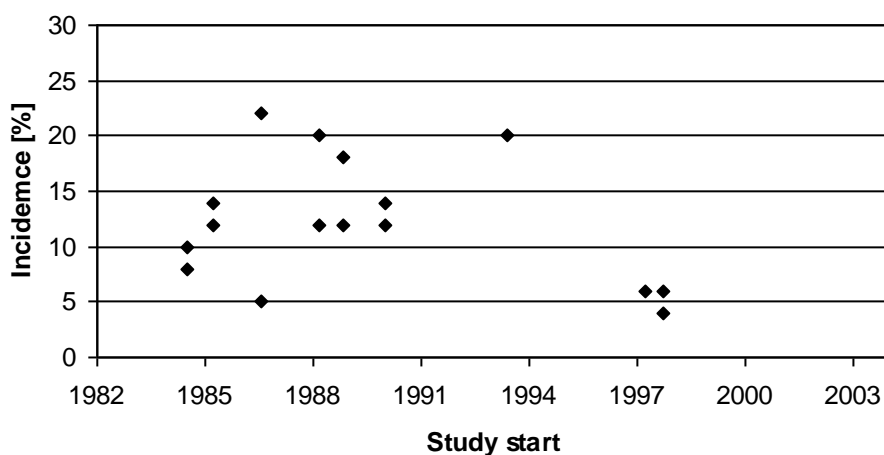


Figure 6: Incidence of LC adenomas in Wistar rats from breeder G: no obvious trend for a decrease with year of study start.

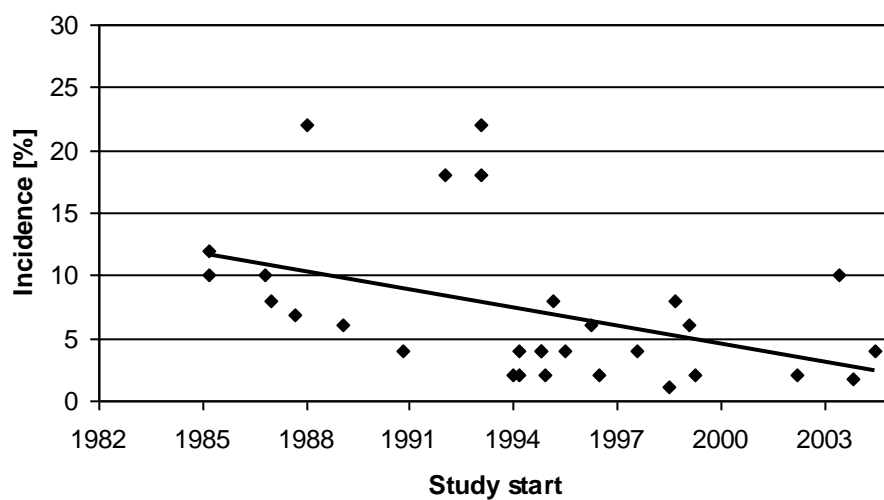


Figure 7: Incidence of LC adenomas in Wistar rats from breeder I: trend for a decrease with year of study start.

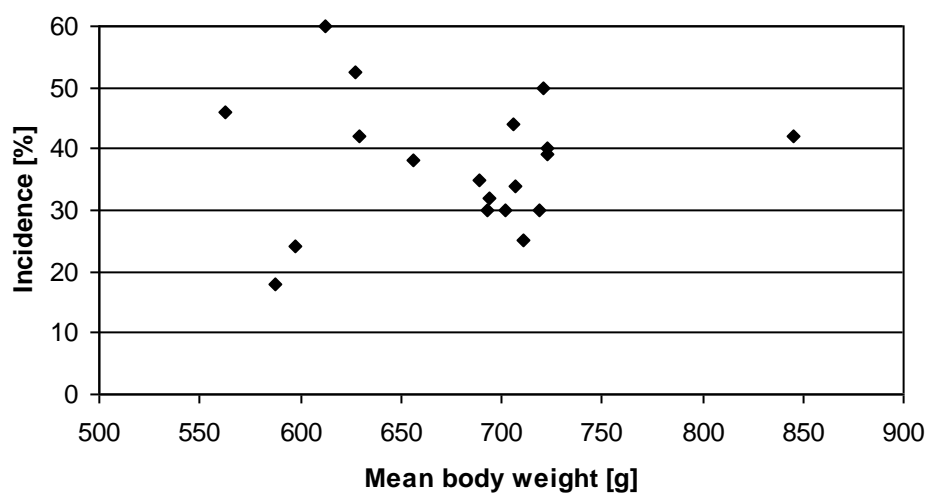


Figure 8: Incidence of LC adenomas in Wistar rats from breeder A: no dependency on mean terminal body weight.

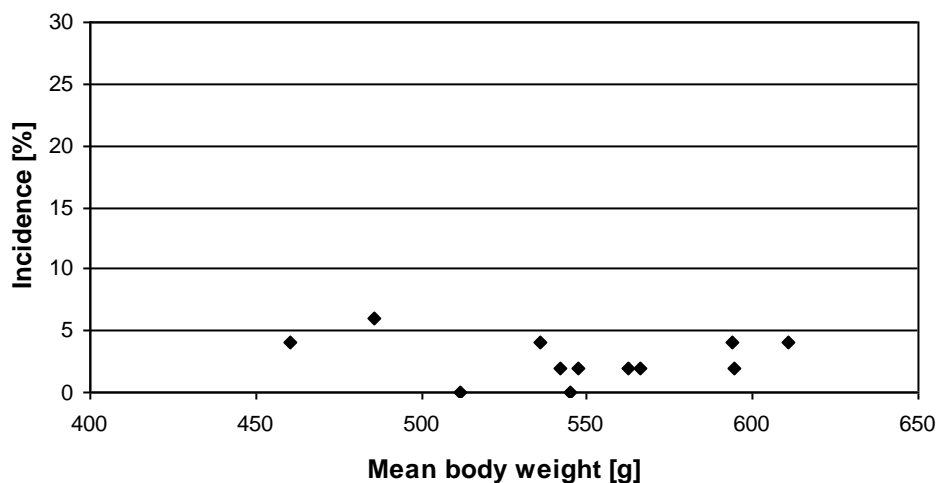


Figure 9: Incidence of LC adenomas in Wistar rats from breeder B: no dependency on mean terminal body weight.

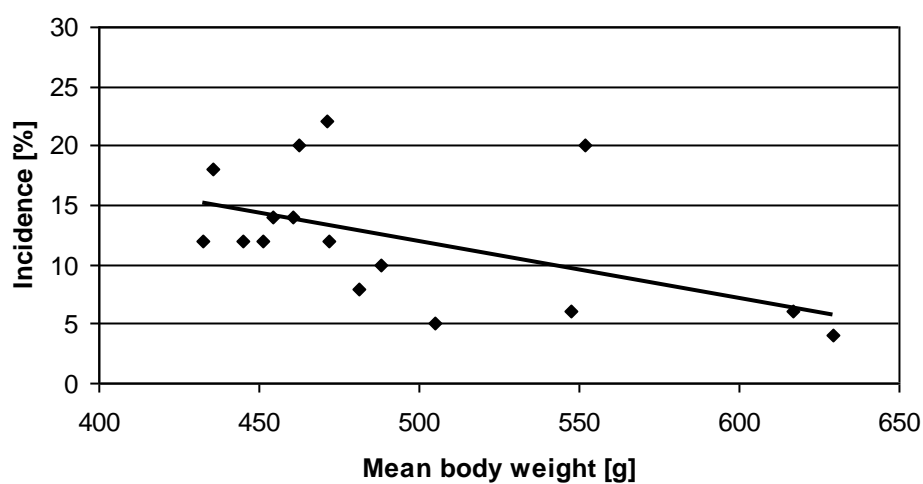


Figure 10: Incidence of LC adenomas in Wistar rats from breeder G: trend for decrease with increasing mean terminal body weight.

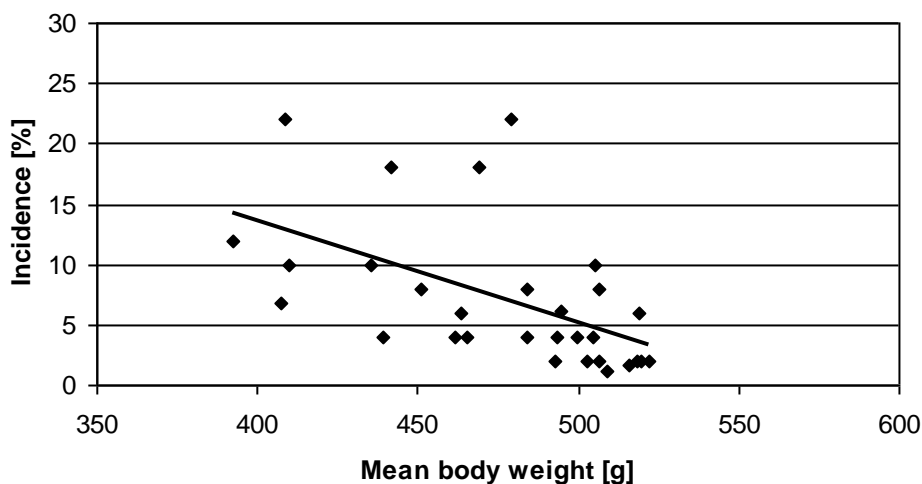


Figure 11: Incidence of LC adenomas in Wistar rats from breeder I: trend for a decrease with increasing mean terminal body weight.

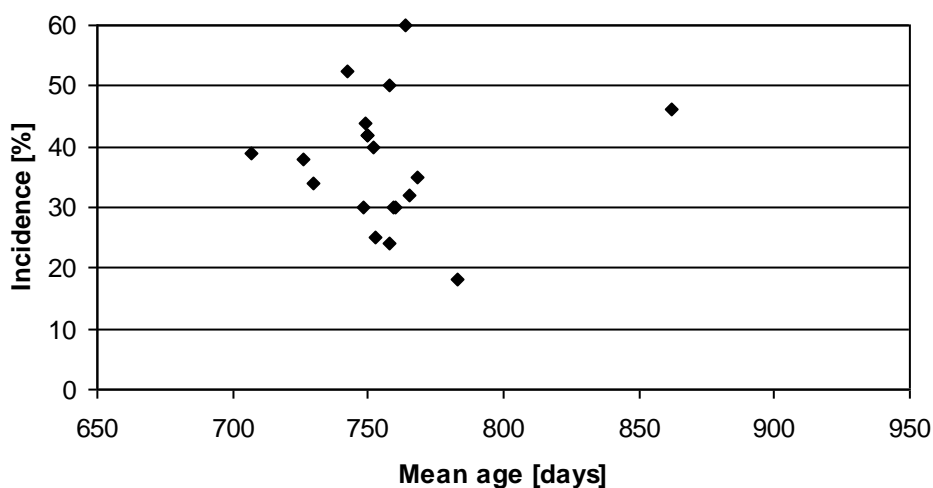


Figure 12: Incidence of LC adenomas in Wistar rats from breeder A: no dependency on mean age at necropsy.

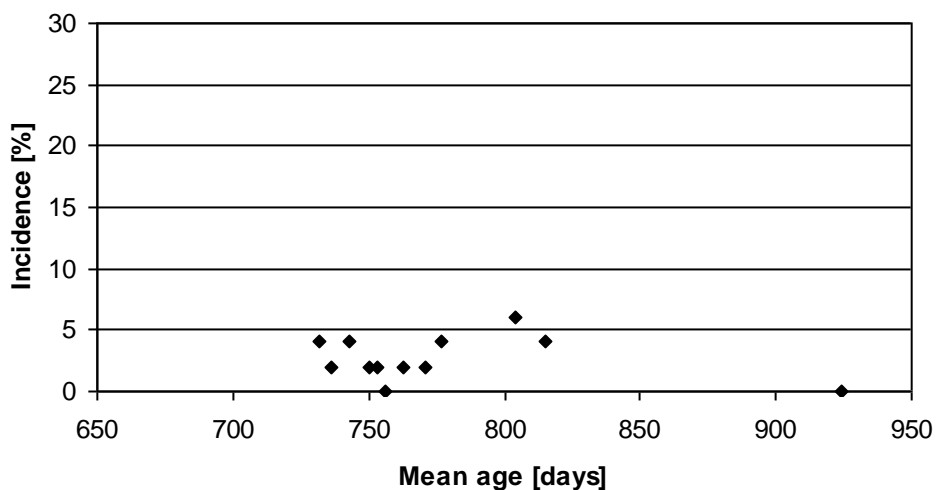


Figure 13: Incidence of LC adenomas in Wistar rats from breeder B: no dependency on mean age at necropsy.

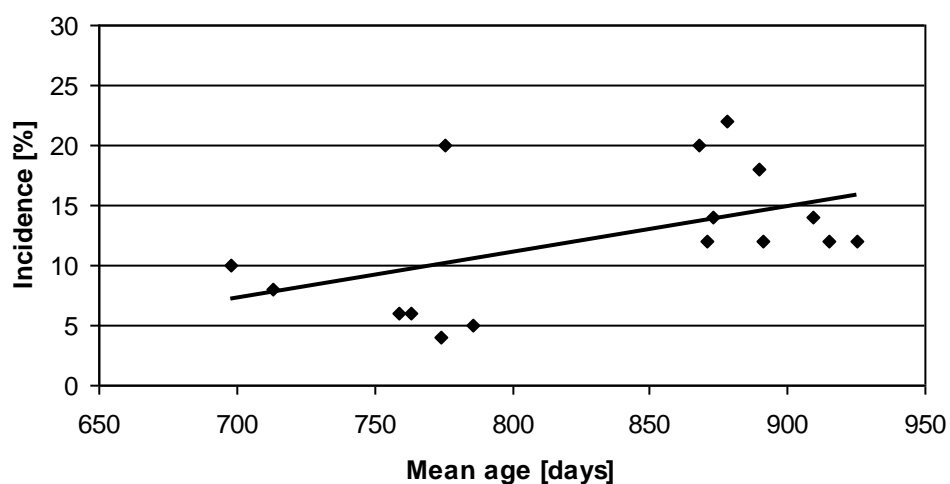


Figure 14: Incidence of LC adenomas in Wistar rats from breeder G: trend for an increase with increasing mean age at necropsy.

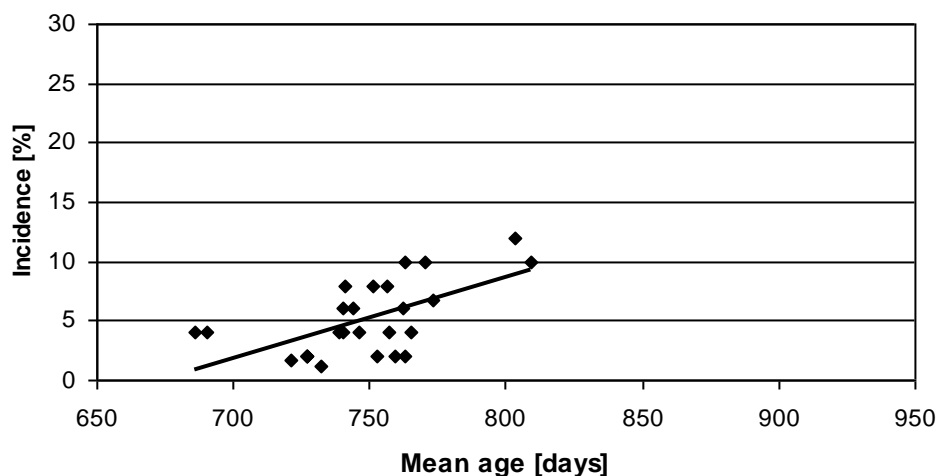


Figure 15: Incidence of LC adenomas in Wistar rats from breeder I: trend for an increase with increasing mean days under test.

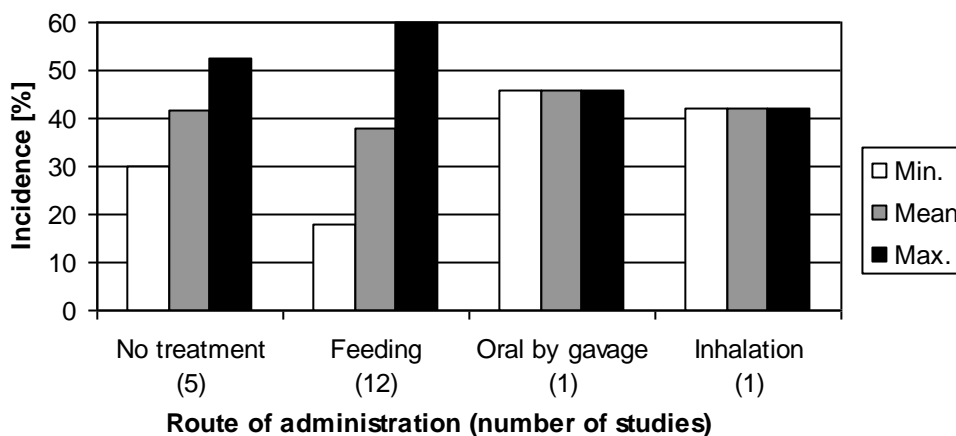


Figure 16: Incidence of LC adenomas in Wistar rats from breeder A: no dependency on route of administration.

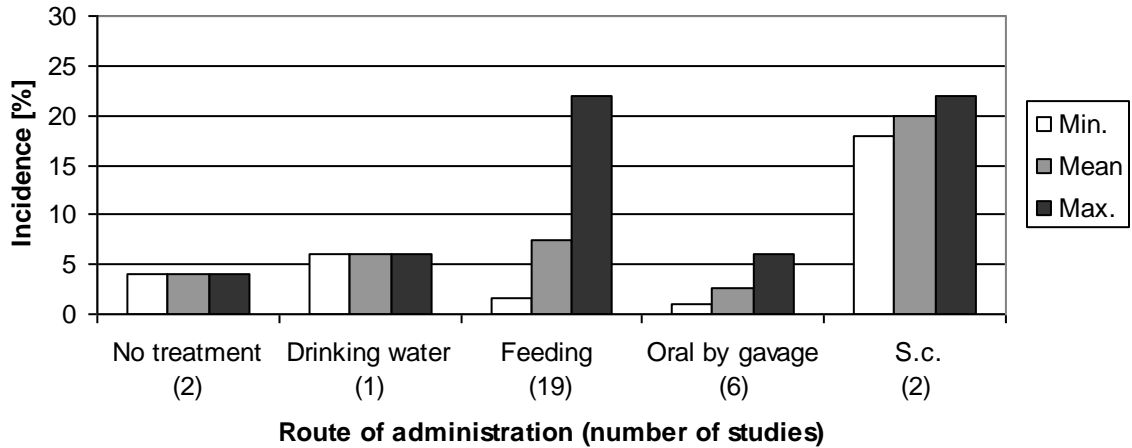


Figure 17: Incidence of LC adenomas in Wistar rats from breeder I: Higher incidence in feeding studies compared to gavage studies. The high incidence in the two studies with subcutaneous injection is not considered to unequivocally indicate an effect of the route of administration (see text).

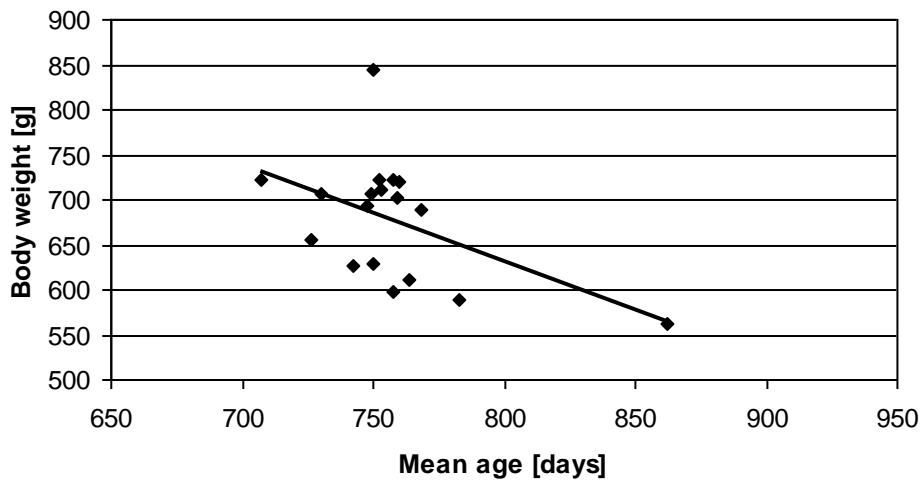


Figure 18: Mean terminal body weight in Wistar rats from breeder A: trend for a decrease with increasing mean age at necropsy.

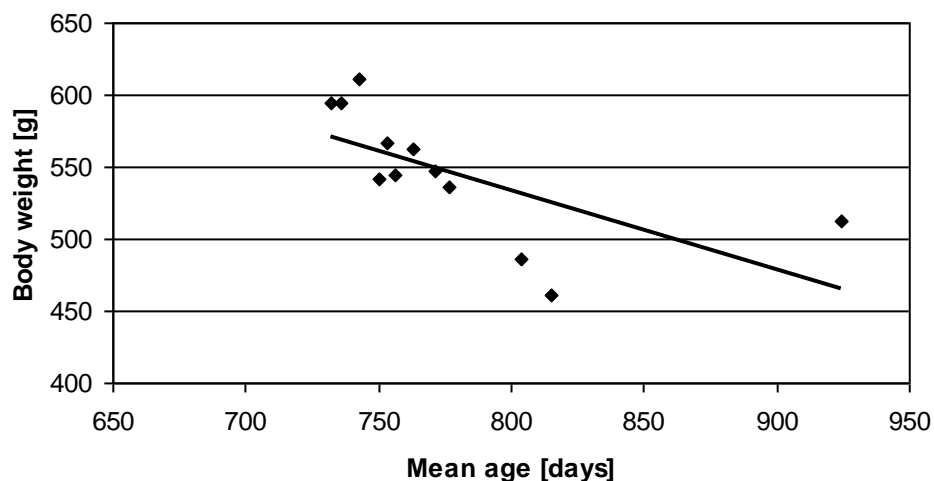


Figure 19: Mean terminal body weight in Wistar rats from breeder B: trend for a decrease with increasing mean age at necropsy.

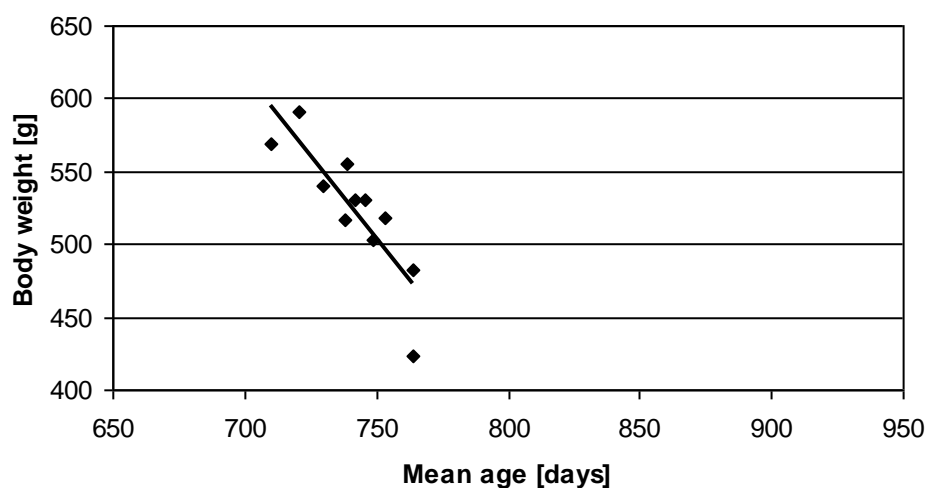


Figure 20: Mean terminal body weight in Wistar rats from breeder D1: trend for a decrease with increasing mean age at necropsy.

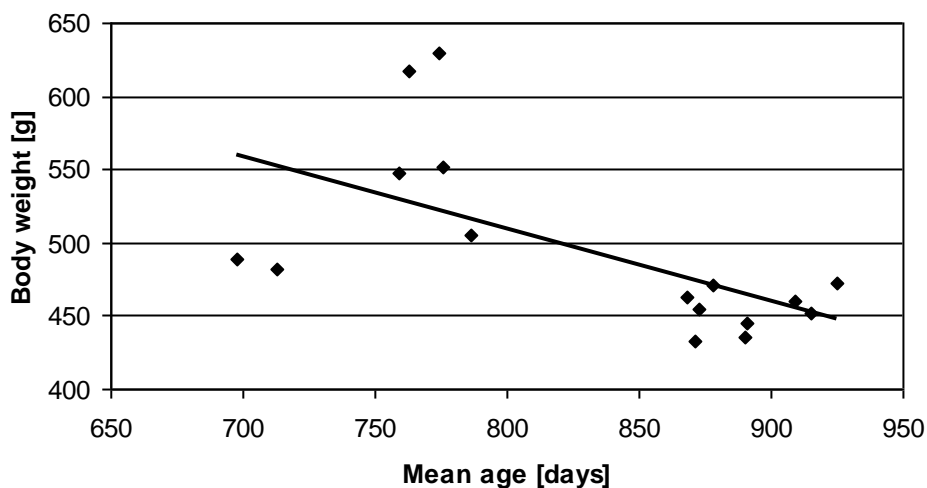


Figure 21: Mean terminal body weight in Wistar rats from breeder G: trend for a decrease with increasing mean age at necropsy.

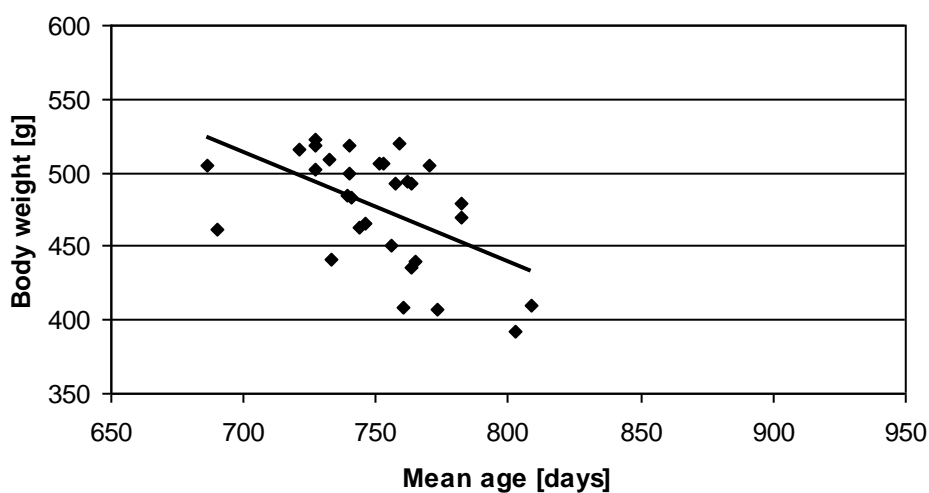


Figure 22: Mean terminal body weight in Wistar rats from breeder I: trend for a decrease with increasing mean age at necropsy.

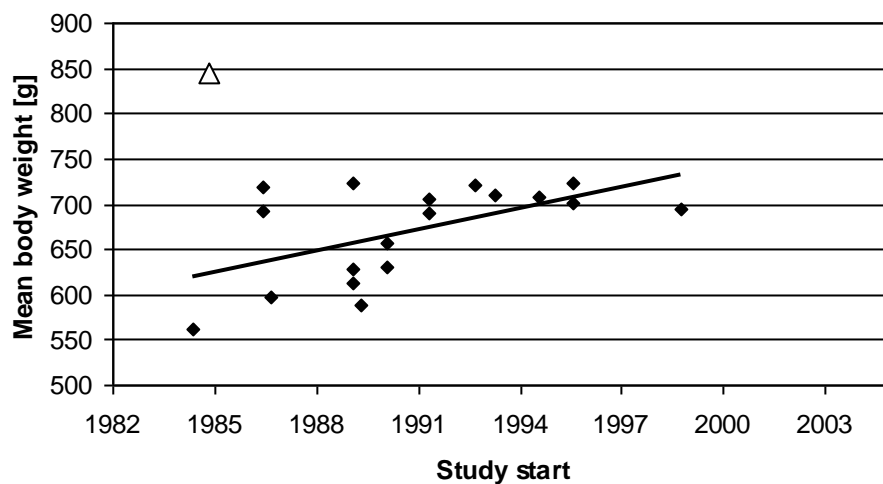


Figure 23: Mean body weight in Wistar rats from breeder A: trend for an increase with year of study start when an extreme value (hollow angle) was excluded.

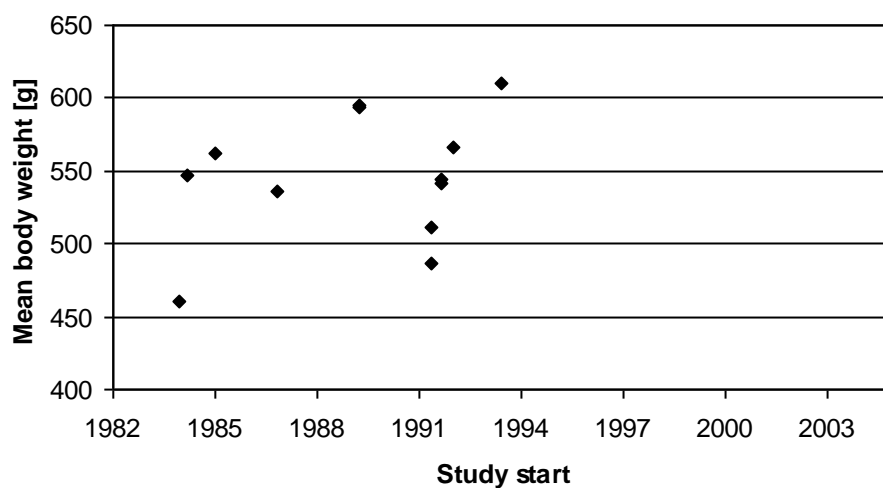


Figure 24: Mean body weight in Wistar rats from breeder B: no clear trend for an increase with year of study start.

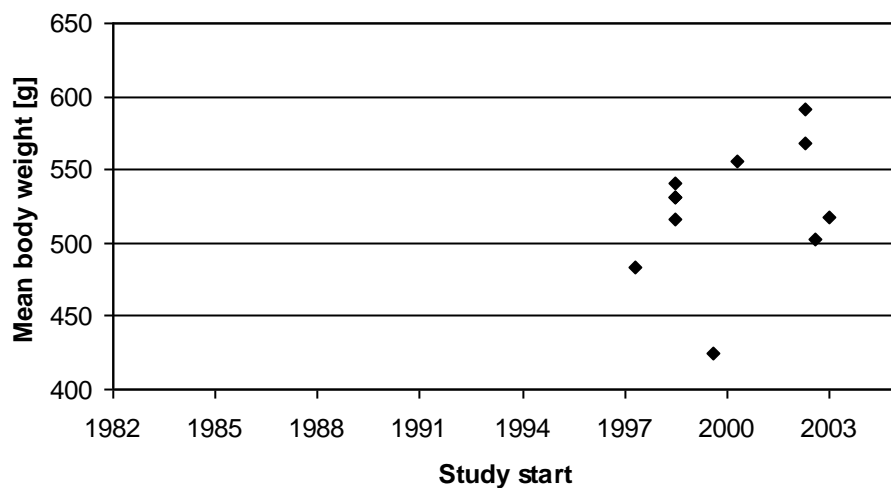


Figure 25: Mean body weight in Wistar rats from breeder D1: no clear trend for an increase with year of study start.

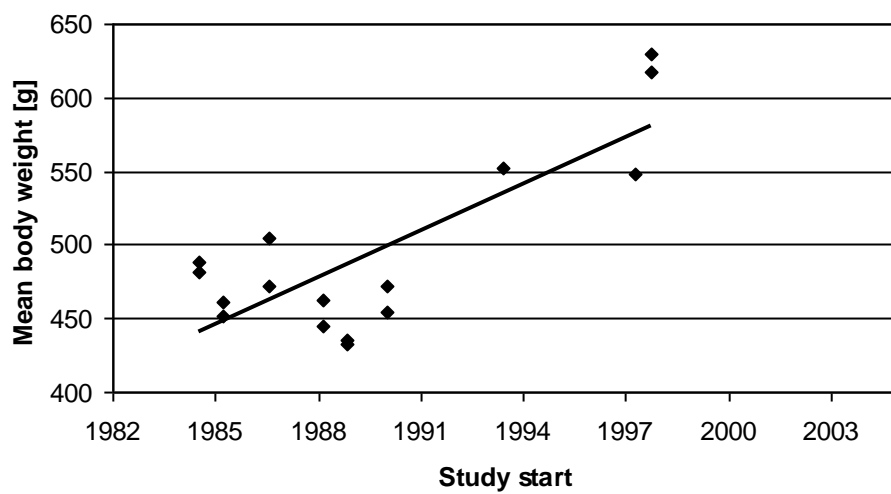


Figure 26: Mean body weight in Wistar rats from breeder G: clear trend for an increase with year of study start.

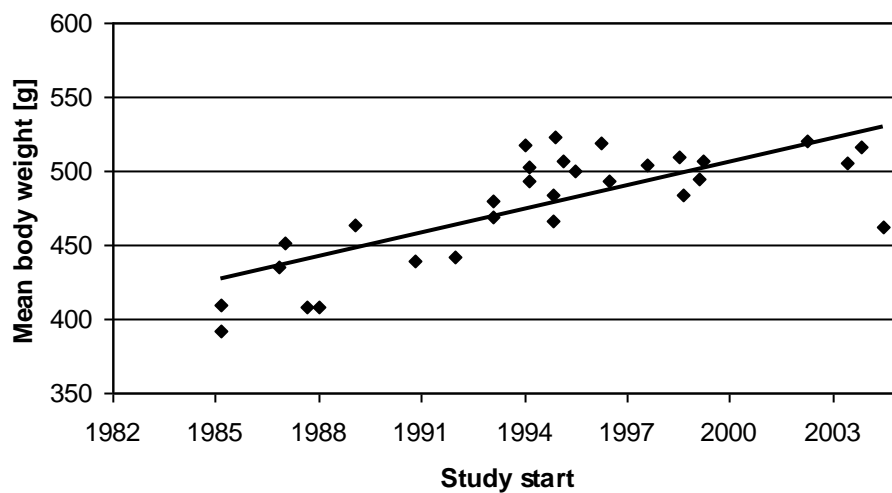


Figure 27: Mean body weight in Wistar rats from breeder I: clear trend for an increase with year of study start.

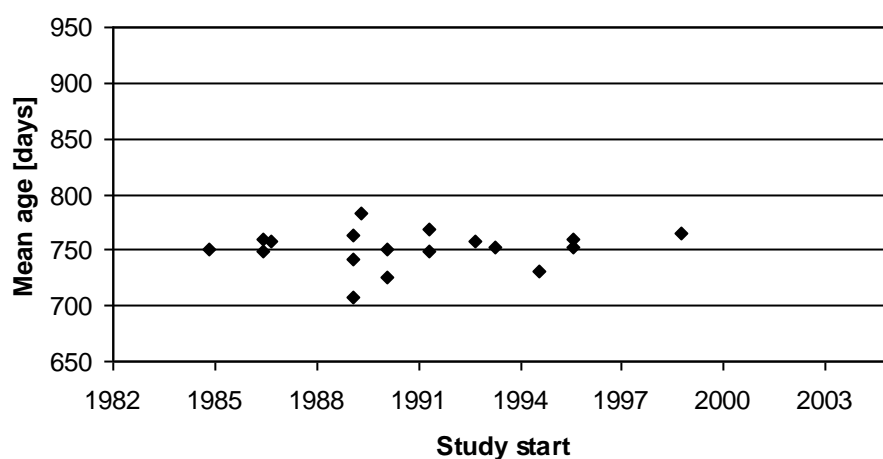


Figure 28: Mean age at necropsy in Wistar rats from breeder A: stability over time.

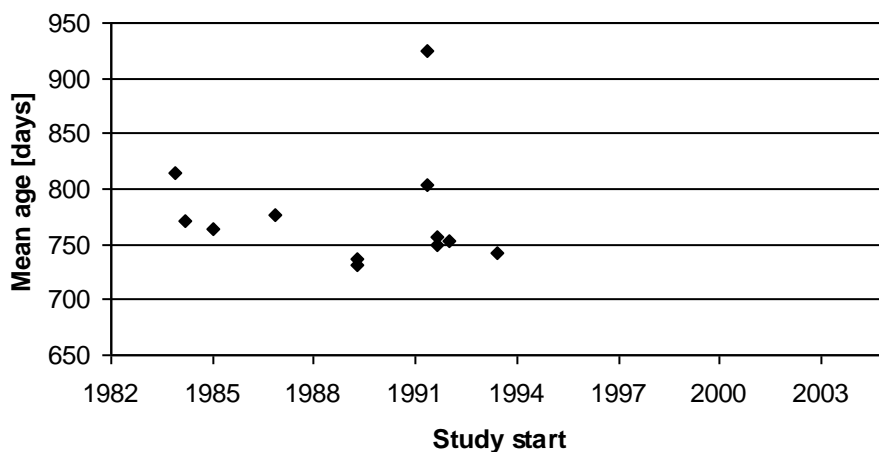


Figure 29: Mean age at necropsy in Wistar rats from breeder B: stability over time.

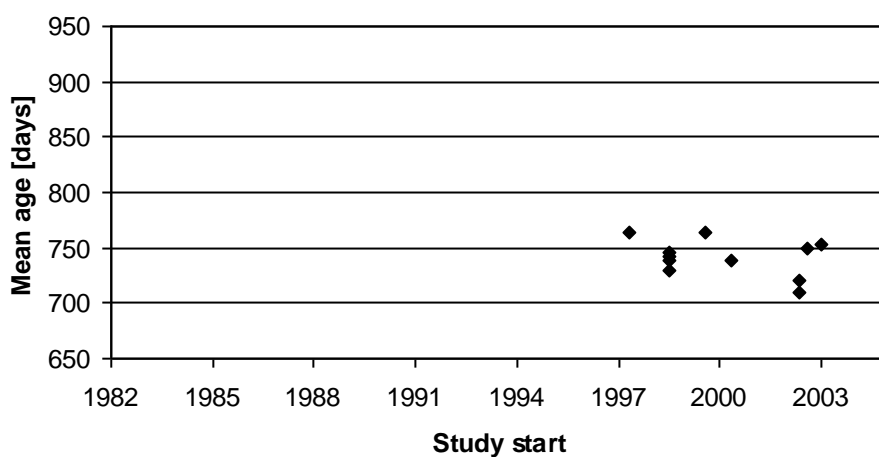


Figure 30: Mean age at necropsy in Wistar rats from breeder D1: weak trend for a decrease with year of study start.

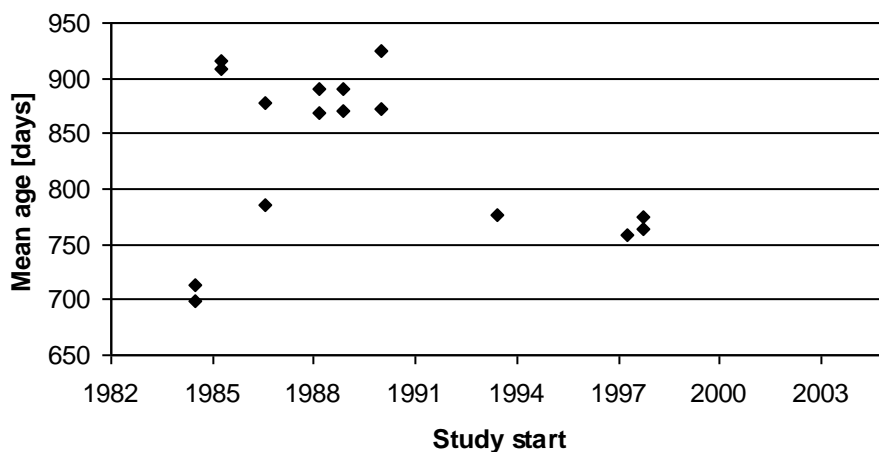


Figure 31: Mean age at necropsy in Wistar rats from breeder G: weak trend for a decrease with year of study start.

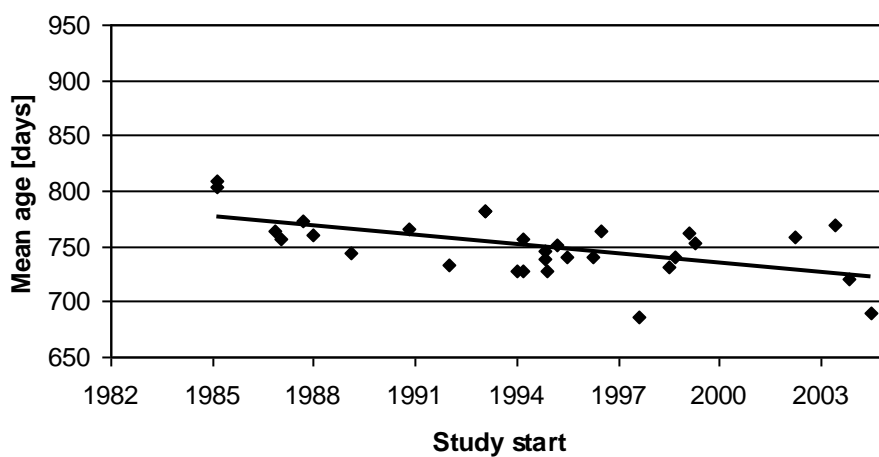


Figure 32: Mean age at necropsy in Wistar rats from breeder G: trend for a decrease with year of study start.