The reproducibility within the laboratory of findings in a three month toxicity study in the rat

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The reproducibility of results from subchronic toxicity studies carried out according to standard protocols is seldom proved. Standard protocols have been developed on long-term experience and are accepted internationally. Sometimes, however, varying test results on the same compound are reported from different laboratories. Such variations are often explained as consequences of interlaboratory differences but the possible contribution of intralaboratory variance is rarely assessed.

For a client the laboratory of Scantox recently conducted a three month rat study twice with the same compound. In the second study a series of additional parameters were examined. This gave the laboratory the opportunity to compare the findings of the two studies and thus evaluate the reproducibility with the time difference between the studies as the main difference.

The studies were conducted according to internationally recognized guidelines for subchronic rat toxicity studies and followed identical protocols apart from inclusion in the second study of extra blood sampling for special analyses. Each study included a control group and three dose groups and each group consisted of 10 males and 10 females. Rats of the strain Mol: WIST were used. For the first study the rats were obtained from 10 litters, each litter consisting of 4 males and 4 females. The rats in each litter were distributed with one animal/sex to each dose group. The compound was administered subcutaneously once daily for 90 days. The first study was initiated in November 1985 and the second in February 1986.

Body weight and food and water intakes were measured once weekly. Food utilization was calculated from body weight gain and food intake data. Blood samples for haematology and clinical chemistry were taken within one day by puncture of the orbital plexus during the week before termination of dosing. The sampling was organized in a randomized sequence in order to avoid diurnal bias. In the first study the samples were taken under light barbiturate anaesthesia and in the second study under carbon dioxide anaesthesia. Conventional methods based on commercially available international diagnostic kits were used. Most parameters determined spectrophotometrically were measured with a LKB 2086 Clinicon AB.

From both studies there appeared a variety of treatment-related changes which are presented in table 1. Apart from a few cases (marked with an asterix in table 1) all changes were exclusively seen in the highest dose group. The increases seen in haematological and clinical chemical parameters did in no case involve a doubling of the level, and the decreases were in most cases less than fifty percent. It is interesting that changes as small as 4-10% attained statistical significance. This indicates that the individual variations of these parameters were very low.

By comparing the direction of changes in both studies there was good correspondance between the majority. For three parameters (monocytes (♂ + ♀), alkaline phosphatase (♂ + ♀) and bilirubin) there was only a tendency towards a similar change as seen in the other study. The percentage of lymphocytes was increased in one study and a tendency towards a decrease was seen in the other study. The percentage of eosinophils was decreased in one study and there was a tendency towards an increase in the other. For calcium, gamma-globulin, potassium and urea an increase or decrease was seen in one study but no change was seen in the other study. A difference in the direction of change was seen with ALAT in males. However, the changes were relatively small; 27% increase in the first study and 18% decrease in the second.

In the majority of three month toxicity studies

TABLE 1. Dose-related changes found in two consecutively conducted three month rat toxicity studies with the same compound. In brackets the percentage of change in high dose group compared to control group.

Parameters	Direction of changes	
	1st run	2nd run
Body weight and food and water intake		
Body weight gain ♂ + ♀	↑	↑
Food intake ♂ + ♀	↑	↑
Food utilization ♂ + ♀	↑	↑
Water intake ♂ + ♀	↑	↑
Haematology		
Haemoglobin conc. ♂	↓ (5)	↓ (5)
Red blood cell count ♂	↓ (10)	↓ (11)
Packed cell volume ♂	↓ (4)	↓ (7)
White blood cell count ♀	↑ (36)	↑ (18)
Monocytes % ♂ + ♀	↓ *(68, 54)	(↓) (50, 60)
Lymphocytes % ♀	↑ (8)	(↓) (4)
Eosinophils % ♀	↓ (64)	(↑) (86)
Clinical chemistry		
ALAT ♂	↑ (27)	↓ (18)
Albumin ♀	↓ (9)	↓ (7)
Alkaline phosphatase ♀	(↑) (17)	↑ *(34)
Alkaline phosphatase ♂	(↓) (18)	↓ (34)
Bilirubin ♂	↑ (73)	(↑) (24)
β-globulin ♂ + ♀	↑ (9, 14)	↑ (9, 25)
Calcium ♂	↑ (8)	- (3)
Cholesterol ♂ + ♀	↑ (97, 62)	↑ (66, 28)
Creatinine ♀	↑ (29)	↑ (15)
Gamma-globulin ♂	- (7)	↑ *(44)
Glucose ♀	↓ (16)	↓ (30)
Inorg. phosphorus ♂ + ♀	↑ *(52, 49)	↑ (24, 48)
Potassium ♂ + ♀	↑ (10, 9)	- (0.3, 5)
Total protein ♂	↑ (7)	↑ (10)
Urea ♂	- (2)	↓ (11)

↑/↓ - statistically significantly increase/decrease ($p < 0.05$, Students t-test)
(↑)/(↓) - tendency towards increase/decrease
\- - no change
* - change in both intermediate and high dose group.

relatively few parameters are affected by the treatment. In the present study unusually many parameters were affected by the treatment and this in particular makes the study useful for evaluation of reproducibility.

When comparing the statistically significant changes it should be kept in mind that the level of significance was 5%, meaning that one out of twenty significances should be expected. According to table 1 fifty-eight statistical significances were obtained.

It is noticeable that the change seen in monocytes and potassium in both sexes in one study was not seen in the other study in any of the sexes. This is interesting since in general changes appearing in both sexes would be taken more seriously than a change occurring in only one sex. It is also noticeable that a tendency towards a change in one study as seen with monocytes, lymphocytes, alkaline phosphatase and bilirubin was statistically significantly changed in the other study. This illustrates that tendency-changes should not always be ignored.

Although different methods of anaesthesia for blood sampling were used for each of the two studies, this should in principle not influence the outcome with respect to drug-induced effect in each individual study because only one method of anaesthesia was used for each separate study. In addition, if the method of anaesthesia should have influenced the outcome, variations would have been expected to occur in both sexes and this was only seen in one case (potassium ♂ + ♀). However, the control level of some of the blood constituents measured in the present studies might fluctuate with the method of anaesthesia used (*Archer and Riley,* 1981) but the level of other parameters than those measured seems to be much more sensitive (*Kraus,* 1980).

It is well known that the concentration of blood constituents is influenced by a number of environmental and methodological factors such as bleeding time, time of the day, different days, light and dark cycle, handling and blood sampling technique (*Archer & Riley,* 1981; *Bickhardt et al.,* 1983; *Gärtner et al.,* 1980). Most of these factors were the same in the present two studies.

Altogether there seems to be a reasonably good correspondance between the findings in the two studies. This adds to the confidence of reproducibility of findings in subchronic toxicity tests conducted according to standard protocols in the same laboratory. The variations between the two studies represent intralaboratory variations which in this case presumably reflect biological variations in the response of the animals. In any experimental study with living

animals this inevitable biological variation should be taken into consideration in the conduct of the study as well as in the evaluation of the results obtained. Although a few variations were seen between the present two studies, the overall safety-related extrapolations to be made would essentially be the same from each of the studies.

Summary

A three month rat toxicity study was conducted twice with the same compound in the same laboratory. The studies were conducted according to internationally recognized guidelines for subchronic toxicity studies and followed essentially identical protocols (a control group and three dose groups, 10 rats/sex/group). The compound was administered subcutaneously once daily for three months. Body weight and food and water intake were measured once weekly. Blood samples for hematology and blood chemistry were taken during the week before termination of dosing.

Body weight gain and food and water intake were increased in both studies. For great many hematological and blood chemical parameters there was a dosedependent statistically significant increase or decrease in one or both sexes. Some changes attained only the level of significance in one study whereas there was a tendency of a similar change in the other study. Only a few deviations (no or opposit change in one study) were seen between the two studies. Altogether there seemed to be a good correspondance between the findings in the two studies and the variations seen would probably not influence the safety evaluation made from any of the studies.

Sammendrag

En tre måneders toksicitetsundersøgelse i rotter blev gennemført to gange med den samme substans i det samme laboratorium. Undersøgelserne blev udført i overensstemmelse med internationalt anerkendte retningslinier for subkroniske toksicitetsundersøgelser og fulgte essentielt de samme forsøgsprotokoller (en kontrolgruppe og tre dosisgrupper, 10 rotter/køn/gruppe). Substansen blev indgivet subkutant een gang dagligt i tre måneder. Legemsvægt og føde- og vandindtagelse blev bestemt ugentligt. Blodprøver til hæmatologiske og klinisk kemiske undersøgelser blev udtaget i den sidste uge af doseringen.

Legemsvægt og føde- og vandindtagelse var øget i den højeste dosisgruppe i begge undersøgelser. For et stort antal hæmatologiske og klinisk kemiske parametre fandtes dosisafhængig statistisk signifikant stigning eller fald hos et eller begge køn i begge undersøgelser. Nogle ændringer nåede kun statistisk signifikans i een undersøgelse mens der var tendens til en tilsvarende ændring i den anden undersøgelse. Kun få variationer (ingen eller modsat rettet ændring i den ene undersøgelse) blev iagttaget mellem de to undersøgelser. Ingen af variationerne syntes at være af en sådan betydning, at det ville have betydning for den sundhedsmæssige vurdering.

Yhteenveto / K. Pelkonen

Kolmen kuukauden toksisuustutkimus rotalla toteutettiin kahdesti samalla aineella samassa laboratoriossa. Tutkimukset suoretettiin subkroonisia toksisuuskokeita koskevien kansainivälisesti hyväksyttyjen ohjeiden mukaisesti ja olennaisilta osiltaan samalla tavalla (kontrolliryhmä ja kolme annosryhmää, 10 rottaa/sukupuoli/ryhmä). Aihe annettiin ihonalaisesti kerran päivässä kolmen kuukauden ajan. Eläimet punnittiin ja niiden ruuan ja veden kulutus mitattiin kerran viikossa. Verinäytteet hematologiaa ja veren kemiallisia tutkimuksia varten otettiin aineen antamisen loppumista edeltävällä viikolla.

Molemmissa tutkimuksissa eläinten ruumiinpainon nousu ja veden ja ruuankulutus lisääntyivät. Varsin monissa hematologisissa ja veren kemiallisissa muuttujissa havaittiin molemmissa tai toisessa sukupuolessa tilastollisesti merkitsevä nousu tai lasku. Eräät muutokset olivat tilastollisesti merkitsevää suuruusluokkaa vain toisessa tutkimuksessa ja toisessa havaittiin suuntaa antavia samanlaisia muutoksia. Ainoastaan muutamia eroja kokeiden välillä voitiin nähdä (ei muutosta tai muutos vastakkaiseen suuntaan). Kokonaisuudessaan tutkimusten löydökset sopivat hyväksyttävästi toisiinsa, eivätkä niiden väliset erot todennäköisesti vaikuttaisi kummankaan tutkimuksen perusteella tehtävään turvallisuuden arvioimiseen.

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