Toxicology and Applied Pharmacology

TNO | Knowledge for business

Didima de Groot[1],
Gert Jacobusse[2],
Jan Lammers,
Andre Wolterbeek,
Stef van Buuren

TNO Quality of Life,
P.O. Box 360,
3700 AJ Zeist,
The Netherlands

1 (neuro)development/toxicology
P +31 30 694 49 54
F +31 30 694 49 86
Didima.deGroot@tno.nl

2 statistics
P +31 71 518 15 96
F +31 71 518 19 20
Gert.Jacobusse@tno.nl

A tool for regulatory safety testing: interval scale for development of rats aged 0-70 days

To protect children against adverse exposure to drugs and chemicals in the environment (including food chain) safety rules have been developed and documented in a number of test guidelines, including those required for experimental testing of potential toxic effects on neuro-development [1,2]. A number of neuro-physical, sensory, motor and cognitive endpoints for rats of different age are included in these test guidelines.

Such regulatory animal studies with extensive behavioural testing are logically complex, labour intensive, time consuming and involve hundreds of animals. Unfortunately, clear guidance on adequate and rational integration of the test results is lacking, mainly because the test scores lack a common metric that allows comparison of developmental scores across age.

Postnatal day 1.

Postnatal day 14: eye opening.

Purpose of the study

- To develop an *interval scale* with quantitative scores, so called *D-scores*, with improved measurement characteristics to estimate normal development in rats using Rasch/IRT analysis [3].
- To use a dataset with many control animals (reference animals) to determine *reference values* for the *D*-scores, i.e. normal development with age.

Relevance interval scale for regulatory developmental/juvenile (neuro) toxicity testing

Once an interval scale for rats is available, the number of tests can be limited by selecting those tests that are most informative to detect delayed/disturbed development. When not limited by strain differences, control groups might be replaced by reference values. For regulatory testing this allows a reduction of labour-intensive testing and reduction and refinement of animal use.

Study design / test system / analysis

- Outcomes of indicators acquired for control rats (aged 0-70 days) during developmental/ juvenile (neuro)toxicity testing will be used to estimate *D*-scores (Table 1).
- As a start, the difficulty was estimated of eight indicators during the first 21 days of life.

Preliminary results of eight indicators and conclusion

Results are presented in Table 2 and Figures 1 and 2.
As expected, the estimations of these 8 indicators show that the difficulty of each indicator agrees with its original test age: indicators tested later at life appear to be the more difficult indicators with higher estimated location.

General discussion on interval scale and *D*-score

Given in separate hand-out

Hand-out belonging to the poster entitled: *'A tool for regulatory safety testing: interval scale for development of rats aged 0-70 days' (Authors: Didima de Groot, Gert Jacobusse, Jan Lammers, Andre Wolterbeek, Stef van Buuren; TNO Quality of Life, Zeist/Leiden, The Netherlands. International Neurotoxicology Conference, 17-21 September 2006, Little Rock, USA).*

Future plans*

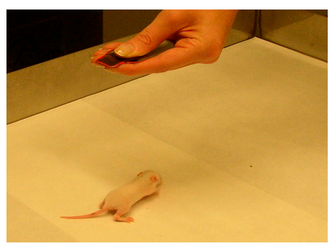
- To ultimately provide a test battery, the analyses with control animals will be repeated for a larger number of tests. Thereby, reference values can be determined which, under specific circumstances, can replace a control group. To provide proper reference values, a minimum of about 500 individuals are required**.
- To investigate the discriminative power of the *D*-score and possibly determine critical values, the *D*-score of a positive control group must be calculated and compared with a control group.

** Financial support requested*

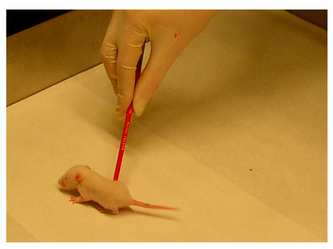
*** Control data of other laboratories requested*

References

[1] Guidelines EPA. OPPTS 870.6300/8600.
[2] Guidelines OECD 426.
[3] Jacobusse *et al*. (2006) Stat Med. 25: 2272-83.



Postnatal day 13: click response.



Postnatal day 13: touch response.

Table 1. Indicators acquired for control rats (aged 0-70 days) during developmental/juvenile (neuro)toxicity testing, used to estimate D-scores.

Table 2. Estimated difficulty of the eight indicators.

Indicator	Test Period on postnatal day	Location	SE
Surface righting	2,3,4,5,6,7	-9.436	0.235
Pinna unfolding	2,3,4,5,6	-9.822	0.242
Hair growth	4,5,6,7,8,9,10	-4.859	0.271
Tooth eruption	9,10,11,12,13,14,15	-0.982	0.250
Click response	13,14,15,16,17,18 etc.	4.171	0.257
Auditory canal opening	10,11,12,13,14,15 etc.	5.559	0.195
Air righting	14,15,16,17,18,19,20,21	7.034	0.207
Eye opening	14,15,16,17,18,19,20,21	8.335	0.233

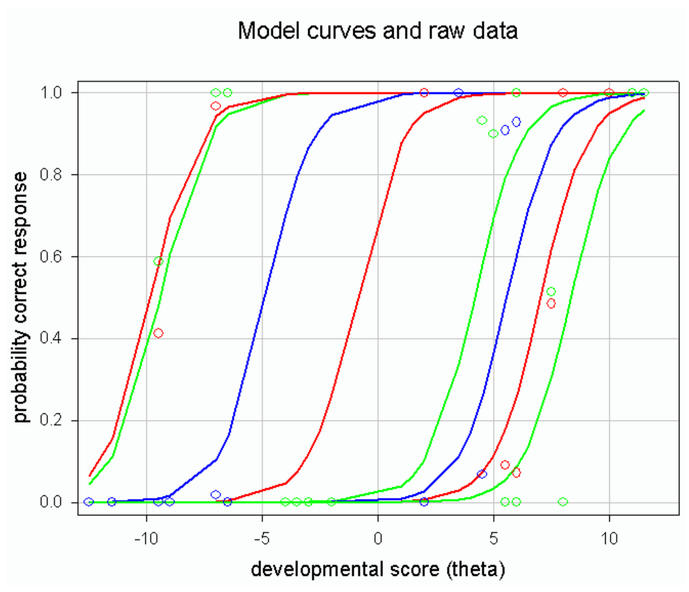


Figure 1. Model fit: estimated curves and raw data.

Figure 1 displays the relation between developmental score (theta) and the probability of a correct response. The locations of the eight curves depend on the location in Table 2. The circles are the raw probabilities of groups of rats with a certain developmental score.

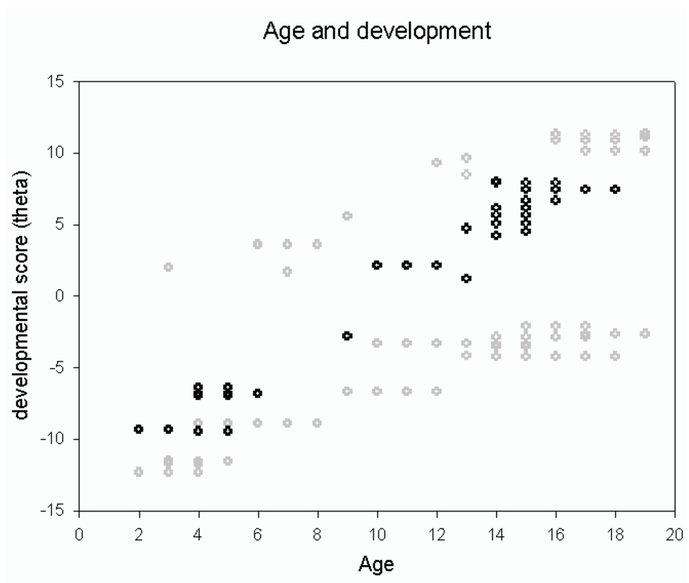


Figure 2. Developmental scores of all rats against age.

Developmental scores of all rats against age are given in Figure 2. Clearly, developmental score increases with age. Within a control group, usual ranges for the developmental score for a given age can be defined.