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A tiered morphological approach (brain size → microscopic slide reading → 2D linear morphometry → 3D stereology) was used in a regulatory developmental neurotoxicity (DNT) study (EPA OPPTS 870.6300) with rats to demonstrate that prenatal exposure to methylazoxy methanol (MAM; doses up to 7.5 mg/(kg day); PN13–15) causes substantial effects on brain morphology, as shown by 2D and 3D morphometry/stereology. During microscopic slide reading the effects went unrecognized (DeGroot et al., 2005a,b). Likewise, significant effects of perinatal exposure to methyl mercury (MeHg; doses up to 1 mg/(kg day); GD6-PD10) were demonstrated, however, by 3D stereology only. Especially total cell numbers and brain region volume showed high discriminative power. So, it was concluded that stereology offers the best opportunity to detect early morphological changes in the developing brain and adverse effects of drugs, chemicals or food components thereon.

However, counting cells is a time consuming job. Instead, volume estimates can be determined fast as long as the investigated region can clearly be defined and borders demarcated. Hereto, we developed a 'novel' mini-atlas of the rat brain defined as 10 major regions. Together with multi-brain embedding/sectioning/sampling, the renewed and *reversed* tiered approach (brain size → 3D stereology → 2D linear morphometry → microscopic slide reading) proved to be sensitive and efficient. The estimates of brain region volumes appeared to be sensitive first-tier indicators to pinpoint the focus for toxicity and to narrow-down the workload. We could demonstrate that stereology is now within reach of cost-effective regulatory DNT testing.

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D16

Regulatory animal testing: Behaviour of rats on a developmental scale with a common metric across age (birth to adultcy)

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The definition of study outcomes is a major problem in regulatory animal testing. In developmental neurotoxicity (Guidelines EPA; Guidelines OECD) behavioural endpoints are commonly measured in rats of different age. However, guidance on rational integration of test results is lacking and it is unclear how these measurements can be combined into aggregated developmental measures. Without such aggregates, the use of the data is inefficient and may lead to multiple testing and incorrect conclusions.

In the present study a new way to deal with this problem is proposed. The central idea is a developmental scale that has a common metric across age (Jacobusse et al., 2006). Both the ability per rat and the difficulty per behavioural task are estimated using a Rasch model. The values on the common scale are called *D*-scores and indicate the ability of the rat to pass a specific task. The strength of the model is that the definition of *D*-scores is not specifically related to age, so that *D*-scores among individuals can be compared independent of age. Differences in *D*-scores between sessions can be used to evaluate the development of the individual animal. The use of *D*-scores in developmental and juvenile neurotoxicity may solve problems associated with the traditional 'per endpoint' testing. Moreover, control groups of the same rat strain need not to be included. Also, the number of tests can be limited using those tests that are most informative to detect delayed/disturbed development, so reducing labour-intensive testing and number of animals.

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