reported of babies born with thalidomide syndrome after the 1960s. Here we reported two additional cases of thalidomide syndrome from the same locality, one born in 2010 and the other in 1998. The first case presented a reduction defect in the upper and lower limbs bilaterally: bilateral absence of the radius and ulna, no thumbs bilaterally; two digits on the right hand and three digits on the left hand; a hypoplastic scapular girdle; bilateral femoral hypoplasia and clubfeet; bilateral bifid hallux, right microphthalmia; and a nevus on her face that was especially apparent on her nose. The mother had ENL and used thalidomide by proxy, without regular contraception. The second case was recognized because of geographic proximity with the first case, and he presented absence of the upper limbs, with pedunculated formation only bilaterally, hypoplastic scapular girdle; asymmetric lower limbs with apparent shortening of the legs, more pronounced on the right; clubfeet with bilateral and asymmetric preaxial polydactyly; hip dislocation; advanced opacification in the left eye iris; dysplastic ears; ear canal stenosis; and ear lobes with a cleft and prominent helix bilaterally. The abdominal ultrasound revealed unilateral renal agenesis. It was confirmed that his mother was being treated for leprosy during the pregnancy. The recognition of these two new cases of thalidomide syndrome emphasizes the importance of pharmacovigilance as well as the importance of studies that attempt to unravel the molecular mechanisms of teratogenic action of this drug in order to generate knowledge for the development of thalidomide analogs without teratogenic effects with consequent reduction of the risk of women exposure in childbearing age.

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Functional assessment of sexual maturity in macaques (*Macaca fascicularis*)

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Introduction: Selection of suitable criteria for assessing sexual maturity in the long-tailed macaque (*Macaca fascicularis*) has been challenging and has yielded conflicting results, in particular for male animals. The establishment of such criteria, however, is pivotal for establishing a solid mating program in order to recruit animals for developmental toxicity studies and, more recently, also in order to take advantage of recent guideline changes related to the use of sexually mature macaques in the context of chronic toxicity evaluation. Whilst in females, menstruation is typically used as endpoint, the situation is less clear for males and body weight, age, hormones and testis size have been used as statistical rather than functional endpoints. This work investigates ovarian cycle criteria for establishing female sexual maturity and whether the presence of sperm in a semen sample can be used to assess sexual maturity in male animals.

Methods: Ovarian cycles were studied by daily vaginal swab analysis. The swabs were rated on a 5-point scale from zero to heavy menstruation and were tracked over several months. Ovarian cycles were tracked and analyzed in more than 1800 animals. For males, 956 animals that provided a baseline semen sample were analyzed for age and body weight and 322 animals were available for correlation of seminal sperm presence and absence with testicular histology at study termination.

Results: Ovarian cycle duration varied between 25 and 35 days in approx. 65% of cycles, and between 20 and 40 days in approx. 30% of cycles. The remaining cycles were considered prolonged or irregular. It was determined that 20 days is the minimal period between two menstruations that was compatible with ovulation and sexual maturity. For males, neither age nor body weight nor testes volume predicted the presence of sperm in semen. In contrast, the presence of sperm in the baseline semen sample correlated with mature testis histology at study termination in every single animal. Surprisingly, in 75% of animals without sperm in the semen sample, spermatogenesis was already mature.

Conclusion: In general, two menstruations at least 20 days apart are compatible with female sexual maturity, and a single semen sample that contains sperm provides functional proof of mature spermatogenesis and, thus, for male sexual maturity.

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Preliminary results of venlafaxine exposure in pregnancy, a multicenter prospective cohort ENTIS study

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Introduction: Venlafaxine (Efexor®) is a serotonin and noradrenaline reuptake inhibitor (SNRI) used for the treatment of depression and anxiety disorders. The limited data on the use of venlafaxine in human pregnancy do not indicate an increased risk of congenital malformations. The main purpose of the study is to assess the rate of major malformations after first trimester exposure to venlafaxine.

Methods: This multicenter, prospective cohort study was performed using data from nine centers who are member of the European Network of Teratology Information Services (ENTIS). Data on pregnancy and pregnancy outcome of women who used venlafaxine in pregnancy were collected during individual risk counseling. Standardized procedures for data collection and follow-up were used by each center.

Results: Follow up data were collected on 744 pregnancies of women who used venlafaxine during gestation. In 583 (78.4%) cases the exposure had occurred at least in the first trimester. In total, there were 600 live births (5 twins), 85 spontaneous abortions, 57 elective terminations of pregnancy, 5 fetal deaths, and 2 ectopic pregnancies.

The overall rate of major malformations after first trimester exposure and excluding chromosomal and genetic disorders was 3.2% (16/500) in all pregnancies ending in delivery, pregnancy terminations or fetal deaths with fetal-pathological examination. Among live births the malformation rate was 2.7% (13/490). We observed no increased risk for organ specific malformations.

Conclusions: The present study indicates that venlafaxine is not a major human teratogen.

Further studies are required to establish its safety during pregnancy.

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Ranking of (neuro)developmental toxicity of organotin compounds in zebrafish relative to their *in vivo* potencies

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The zebrafish embryotoxicity test is an alternative test system for relative fast and simple screening of the embryotoxic properties of chemicals. Furthermore, zebrafish locomotor activity and especially total distance moved as a measure for developmental neurotoxicity can be easily and rapidly measured in larval zebrafish using an automated video image analysis system. However, these assays are only useful if they predict the (neuro)developmental potential of chemicals comparable to observations in in vivo developmental toxicity studies.

In this study, a group of organotin compounds known to induce general development effects and/or neurodevelopmental effects in studies with rodents were evaluated in zebrafish. Hereto, various different organotin compounds (mono-, di and trimethyltinchloride and mono-, di- and tributyltinchloride) were administered to zebrafish embryo at various concentrations. At 24, 48, 72 and 96 h post fertilization (hpf), various morphological and physical parameters were scored to assess lethality and/or developmental effects. At 100 hpf, all viable larvae were used to assess locomotor activity using a video-tracking system (View-point) and subsequently the larvae were processed for microscopical examination and for substance uptake determination. In the embryotoxicity assay, dose-dependent effects were observed on hatching and other abnormalities. In the motor activity assay, the most sensitive parameter was total distance moved and a dose-related hypoactivity was frequently observed. Furthermore, microscopic examination also revealed histopathological changes in the brain of the larvae. When comparing the results of this study to in vivo data, the potency ranking of the compounds for developmental toxicity and neurotoxicity was comparable to their in vivo ranking. These results show that the zebrafish model is a promising model for the prediction of toxic potencies and a useful tool to study biological response similarities of structurally related substances.

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Integrated testing strategy for reproductive toxicity

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The current system of risk assessment of chemicals is complex, very resource-intensive and time-consuming which will become even more clearly by implementation of the REACH regulations. Within these regulations, the requirements for reproductive and developmental toxicology are particularly important since these studies require most resources. Therefore, there is a great need to modernize the process or hazard- and risk assessment, requiring registrants to consider alternative methods of filling the data gaps. Since the complexity of the reproductive and developmental processes, the use of alternative methods for these endpoints may be problematic. At this moment, there are only a few alternative methods identifying potential reproductive toxic agents with sufficient accuracy, speed and reliability. Simple animal-free *in vitro* models cover only a restricted part of the reproductive cycle. Most models represent underlying processes and dynamics insufficiently and are therefore of limited use as a stand alone.

The EU project ChemScreen aims to fill these gaps and place the tests in a more innovative animal free integrated testing strategy for reproductive toxicity, which will use combinations of available *in silico* and *in vitro* technologies.

A first step in the project is to establish methods for prescreening and prediction of chemicals having specific toxicological properties that do not need further testing for reproductive toxicity according to REACH, i.e. chemicals that need classification as either genotoxic carcinogen or germline mutagen. In this step also methods for prescreening and predicting potential reproductive toxicity using repeated dose and reproductive toxicity databases and *in silico* methods are envisaged. A minimal set of medium- and high-throughput *in vitro* test methods to study sensitive parameters will be established as a second step to identify reproductive toxicants. For the short run these methods will be applied and tested for use in a category approach to verify the read across to an *in vivo* tested member, while the long run objective is to develop them into a stand-alone battery. In the final step all this information is integrated to allow conclusions on classification and labeling and risk assessments to be made, among others by applying quantitative *in vitro-in vivo* extrapolation, and herewith to decide on the need for and specifics of further *in vivo* testing for reproductive toxicity.

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Differential performance of Wistar Han and Sprague Dawley rats in behavioral tests: Differences in baseline behavior and reactivity to positive control agents

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In studies for general toxicity assessment, Wistar Han (WH) and Sprague Dawley (SD) rat strains are both acceptable for EU and US test guidelines, though WH are utilized preferentially in Europe and SD rats are generally preferred in the US. Despite the acceptability of both the WH and SD strains for toxicity testing, specific differences between the strains have been demonstrated in some endpoints, though disparities are not always seen. Developmental neurotoxicity (DNT) testing assesses potentially adverse effects on the developing nervous system. The present DNT study was conducted to generate historical and positive control data using the commonly used WH and SD strains. Potential differences between these strains in DNT endpoints have not been extensively investigated. Behavioral tests including motor activity, startle response, learning and memory testing as well as neurological examinations, including both quantitative and qualitative assessments, were conducted. Three groups were used including control, prenatally exposed (to Methylazoxymethanol [MAM] on gestation Day 15) and acutely-treated animals (with IDPN, MK-801 or Chlorpromazine) for each strain. There were limited functional differences seen between the strains in baseline behavior, or in sensitivity to most of the positive controls used. Furthermore, the positive controls produced clear effects in most endpoints investigated. For both strains,