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Postnatal fate of prenatal-induced fetal alterations in laboratory animals

Thomas Hofmann*, Roland Buesen, Steffen Schneider, Bennard van Ravenzwaay

BASF SE, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany

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ABSTRACT

Currently it is common practice to evaluate the developmental toxicity hazard of chemicals or pharmaceuticals by evaluation of fetuses after administration of the compound to pregnant animals. These studies are designed to provide possible compound-related fetal changes near term, which are usually classified into malformations or variations. Malformations, but not variations are expected to adversely affect the survival or health. Therefore, classification has striking different regulatory consequences. For categorization as variation reversibility is an important criterion, but it is usually not examined in a standard guideline study. Although this issue has already been recognized long time ago, data dealing with the postnatal reversibility of fetal alterations are still rare. In the current review, literature data, regulatory documents as well as in-house data were compiled. Beside skeletal alterations of skull, vertebral column, ribs, shoulder and pelvic girdle, and extremities, kidney and heart defects are discussed and assessed.

1. Introduction

Determination of the developmental toxicity hazard of chemicals or pharmaceuticals is usually made by administration of the compound to pregnant animals with possible exposure of the conceptuses via the placenta (e.g. OECD414 [1], ICH [2]). Testing is usually performed in rats (rodent) and rabbits (non-rodent), in certain cases also in mice, hamsters, guinea pigs, minipigs, monkeys and sometimes other species. After cesarean sections shortly before expected birth, fetuses are examined for external, visceral and skeletal abnormalities. Based on this design, these studies provide a snapshot into possible compound-related fetal changes near term. However, they do not allow a statement concerning postnatal fate of prenatally induced alterations.

Fetal pathology changes are usually classified into malformations and variations, which are sometimes also described as major and minor anomalies. A malformation is defined as 'a permanent structural change that is likely to adversely affect the survival or health of the species under investigation' [3]. In contrast, a variation is defined as 'a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health' [3]. In earlier times, the classification into these both categories was not uniformly done. Therefore, the so-called 'Berlin Workshops' [3–5] were held with the aim to harmonize classification and nomenclature of fetal alterations as either malformation or variation on an international level. However, a number of findings still remain, for which a clear assignment into the two categories has not be made (so-called grey-zone anomalies). Particularly for those, but also for other findings which have been classified into either of the two categories, information on postnatal reversibility/irreversibility is essential to ensure proper classification.

Classification of fetal alterations has substantial consequences in a regulatory framework for both labelling and risk assessment. In Europe, compounds classified as developmental toxicants in Category 1A (known human reproductive or developmental toxicants) or 1B (compounds causing reproductive or developmental toxicity in well-conducted animal experiments, not secondary to maternal toxicity) cannot be approved according to the Plant Protection Product Regulation Directive unless its exposure to humans under realistic conditions of use is negligible [6]. A similar situation exists in the authorization of chemicals under the REACH legislation (Substances of Very High Concern) and in the Biocidal Product Regulation [7,8].

Looking at the definitions, malformations are defined as permanent changes. However, it is sometimes difficult to judge whether or not a change is permanent, since the postnatal fate of prenatally induced alterations is often not followed up. Although this issue has already been recognized more than 30 years ago [9], data deal-

Abbreviations: GD, Gestation Day; PND, Postnatal Day; VSD, ventricular septal defect.

* Corresponding author.
E-mail address: Thomas.b.Hofmann@basf.com (T. Hofmann).

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ing with the postnatal reversibility of fetal alterations evaluated at cesarean section are still rare.

The aim of the present article is therefore to compile the available data in the literature as well as to identify data gaps. Evaluated data consisted of literature and regulatory reports. It should be noted that functional impairments are usually not evaluated by these study designs. Therefore, the present paper focuses on variations and malformations.

2. Evaluation of available data—skeletal findings

The following text is ordered from the skull to the toes.

2.1. Skull

The bony part of the skull can be divided in the neurocranium consisting of the brain capsule and the chondrocranium (base of the skull), and the viscerocranium, which forms the face. The viscerocranium develops from the first and second pharyngeal arch. The mandibular arch forms the mandibular and maxillar process. The maxillar process differentiates into malar bones, maxilla and part of the parietal bones, whereas the mandibular process forms mandibular, Meckel's cartilage and – together with the second pharyngeal arch – the ossicles. The vast majority of the skull bones originate from neural crest cells. Craniofacial defects like cranioschisis (closure defect of the neuroporus cranialis leading to anencephaly, exencephaly or encephaolcele) are generally irreversible. These defects are induced by e.g. retinoic acid due to imbalances of neural crest cell differentiation [10]. The same counts for calvarial craniosynostoses, premature closure of calvarial sutures which compromise flexibility of the skull during postnatal development and lead to misshapen skull bones.

Literature concerning the postnatal development of skull anomalies is scarce. Beck [11,12] examined mice, whose mothers were treated intraperitoneally with Trypan Blue on gestation day (GD) 7–9, on postnatal day (PND) 62 (two experiments). Interfrontal bone was present in more than 66% and 68% of these mice, compared to 18 and 11% in controls. However, the question remains if this anomaly represents an adverse effect. In a similar study design, 28 days old mice, whose mothers were treated orally with 2,4,5-trichlorophenoxyacetic acid from GD 6–15, showed fused frontal bones at an incidence of 13.7%, compared with zero incidence in the untreated control and vehicle treated group [13]. Increased incidences of parted frontal bones (72% versus 45%) and accessory parietal bones were present in 63 days old offspring after treating the mothers subcutaneously with acetazolamide on GD 8 [14]. These findings suggest that these alterations are permanent, but nevertheless may classified as variations based on the high spontaneous incidence in this case.

Tokioka et al. [15] examined the development of the maxilla and zygomatic bone as well as the fusion of the two skeletal elements in rabbit newborns and up to 4 weeks thereafter. In newborns the zygomatic process of the maxilla and the zygomatic bone were not connected yet, while at an age of one week the anterior end of the maxillary process and the posterior end of the zygomatic bone were starting to merge along their edges, forming a zygomaticomaxillary suture. At that age the two bones were already no longer separable. The fusion was complete at an age of 4 weeks when almost the entire suture line had disappeared. It can be assumed that this process takes place in other species in a similar way, although the age when the sutures connect may differ significantly from species to species. Our own data show that in Wistar rat offspring the zygomaticomaxillary fusion is complete not later than at an age of 3 weeks, and no fissure or suture is discernible in this specimen. In 5–8% of rat fetuses the fusion is present shortly before birth [16,17]. In Rhesus monkeys the sutures of the zygomatic with its adjoining skull bones (including maxilla) may persist lifelong. Variation in the age of fusion of each adjoining bone was large and the frequency of complete zygomaticomaxillary fusion was between approximately 15% in females and 25% in males of the investigated sample [18]. Also in humans the two bones merge, but zygomaticomaxillary sutures may persist into adulthood [19]. Taking these species differences into account it seems not of vital importance for the later growth and stability of the skull in general whether zygomatic and maxilla completely fuse or zygomaticomaxillary sutures persist. Thus it is questionable whether the zygomaticomaxillary fusion in rat term fetuses is a variation at all or if it should just be noted as unclassified observation.

2.2. Vertebral column

In all mammalian species the segmented vertebral column and related structures are formed by segregation of the unsegmented paraxial mesoderm into metameric somites [20]. In rats this process starts on GD 9.5 [21]. The dorsal part of the somites then differentiates into the dermomyotome and the ventral part into the sclerotome. The dermomyotome further develops into the dermis of the back and the skeletal muscles. The ventromedial cells of the sclerotome cells form the vertebral bodies and intervertebral disks, whereas the lateral part of the sclerotome is involved in the formation of the ribs, the vertebral arch and the connective tissue around the dorsal root ganglia. The caudal part of each sclerotome segment fuses with cranial part of following segment (re-segmentation). At the end, each vertebra consists of the caudal half of one somite and the cranial half of the adjacent somite [22]. Errors in the process of re-segmentation result in the formation of anomalies of the vertebral column (scoliosis).

Most frequently recorded anomalies of the vertebral column are supernumerary, absent, split or fused vertebrae, misshapen vertebrae and hemivertebrae. The latter changes lead to the clinical picture of scoliosis [23]. All these anomalies are usually considered to be permanent and, therefore, should be classified as malformations. In addition to these structural changes altered ossification is often observed in toxicological studies. Ossification of the vertebral centres begins with two separated ossification centres on the left and right side of the vertebral centre on gestation day 17–18 in rats and on GD 20 in rabbits [24,25]. These centres fuse during later development, forming first dumbbell-shaped vertebral bodies and finally a uniformly ossified vertebral body [26]. In case of delayed development, cleft or dumbbell-shaped vertebral bodies are diagnosed in prenatal toxicity studies at cesarean section. These changes are usually classified as variations, since postnatal ossification is principally expected.

An examination of the postnatal development of these changes in offspring in rats, which were induced by administration of the anti-metabolite 5-fluoro-20-deoxyuridine to their mothers, was performed on PND 7 and 21 [17,27–29]. Whereas incidence and severity of findings were higher at cesarean section, dumbbell-shaped vertebral bodies were still present on PND 21. These data suggest a partial, but not complete reversibility of this finding by PND 21. In another study, pregnant rats were treated with ethylene glycol (which causes maternal acidosis) from GD 6–15. Incidence of skeletal anomalies were examined on gestation day 18 and 20, as well on PND 1, 4, 14, 21 or 63 [30]. Persistence of bipartite or dumbbell shaped vertebral bodies on day 21 after birth was confirmed in this study, but the incidence dropped near to zero on PND 63. Cartilage was present around the ossification centres on PND 21, which enabled subsequent ossification. Taken together, these results indicate that bipartite or dumbbell-shaped vertebral bodies are principally transient and reversible, provided that the appropriate cartilage model is present. However, the ossification process