CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DEC 12 1985

MEMORANDUM

SUBJECT: EPA Reg No. 8340-15: Teratogenicity studies with Triphenyltin Hydroxide - Receipt of Reprint Discussing Significance of Hydronephrosis as a Teratogenic Lesion in Rats.

TOX CHEM No. 896E

FROM: John Doherty 12/11/85
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: Henry Jacoby
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Registration Division (TS-767)

THRU: Edwin Budd
Section Head
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Hazard Evaluation Division (TS-769)

12/9/85
12/11/85

In a previous memo Toxicology Branch (TB) requested that a specific reference which the registrants used to defend their position that triphenyltin hydroxide is not teratogenic in rats be provided to TB for evaluation (refer to J. Doherty memo dated August 8, 1985 for EPA Reg. No. 8340-15).

The registrants have complied with this request and submitted a reprint entitled "Common Fetal Aberrations and Their Teratologic Significance: A Review" by K.S. Khera as published in Fundamental and Applied Toxicology 1:13-18 (1981). TB acknowledges receipt of this reprint (attached).

With regard to the problem of possible potential teratogenic effects of TPTH based on hydronephrosis, TB notes that this author considers that the presence of hydronephrosis (and certain other lesion types) when it occurs in the absence of frank teratogenic responses is "hard to interpret". It is im-

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plied by this article that hydronephrosis may or may not represent "something of a pathological significance". The article also concludes that "the cause and significance of fetal aberrations [including hydronephrosis] must be established before teratogenic studies can be more precisely interpreted".

With the comments of this reprint taken into consideration, TB feels justified in its prior requests to conduct teratology studies with rats which included postnatal development assessment.

TB has recently determined that TPTH is not teratogenic in rats (refer to the memo entitled "Triphenyltin hydroxide: Rebuttal comments to PD 1 and request additional immunotoxicity studies" dated November 6, 1985 and addressed to B. Shackleford).

TB thanks the registrants for submitting this reprint. This paper is useful in assisting in the review of teratology studies especially related to studies which may show increases in lesions such as hydronephrosis, ossification of the sternum, and the presence of supernumary ribs.

FUNDAMENTAL AND APPLIED TOXICOLOGY 1.13-18 (1981)

Common Fetal Aberrations and Their Teratologic Significance: a Review*

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ABSTRACT

Common Fetal Aberrations and Their Teratologic Significance: a Review. Khera, K.S. (1981). *Fundam. Appl. Toxicol.* 1:13-18. Fetal aberrations occurring at a greater incidence in the test than in the control groups make it difficult to estimate human safety from animal data. This is due to lack of agreement on the teratologic significance of aberrations and our ignorance of mechanisms governing their appearance. Quite often the aberrations are transitory and tend to disappear on further development. A requirement for specially designed studies exists in order to determine the true nature of aberrations and their effects on postnatal development. Only then will it be possible to establish a scientific basis for using animal data to estimate human safety. This review will illustrate the need with examples: changes in the ossification of sternum (which may be retarded by factors remotely related to a test chemical), presence of hydronephrosis (which cannot be diagnosed accurately owing to extreme physiological variations in size of the renal pelvis) and presence of supernumerary ribs (a fetal variation which has been inconsistently interpreted). Relatively little is known about the significance of bent or wavy ribs and undescended testes. Future research should be directed towards determining the teratologic significance, if any, of these and other embryological deviations occurring in experimental animals.

INTRODUCTION

Fetal aberrations consist in minor structural changes, distinct from malformations and are tentatively divided into retardations, variations and deviations (Fig. 1). Retardations are regarded as provisional delay in growth or morphogenesis, which has otherwise followed a normal pattern of development. A retardation may be general or local depending on whether the whole or part of a fetus is involved. Retardation, generally considered a norm, can be manifested in several ways including: 1) incompleteness of ossification as it is seen in sternebrae, vertebrae and skull bones (Perraud, 1976), 2) delayed development, such as increased dilation of renal pelvis bordering on hydronephrosis, and 3) slowed migration of certain organs or parts, for example abnormal descent of testes from the abdomen to the scrotum.

Variations, considered hereditary, are controlled by complex factors both genetic and extragenetic. A genetic basis influenced by unknown determinants, has been postulated for variations in the vertebral column of man and swine, and in the number of ribs and the position of the sacrum of rabbits (Sawin, 1937; Green, 1939).

Deviations are minor changes arising from alterations in the differentiation. These are either transitory tending to disappear within a reasonable time, or permanent but relatively innocuous, without any visible alteration in external morphology, functional activity or survival of the fetus.

Malformations in term fetuses observed during teratologic screening are defined as anatomical anomalies that, based upon prior knowledge derived mostly from postnatal studies, have been accepted as incompatible with survival (at and beyond parturition), growth, development, fertility and longevity.

A distinction between aberrations and malformations, based only on observations of anatomical changes of term fetuses may at times become extremely difficult, since the effects of aberrations on postnatal development have not been systematically investigated.

The incidences and kinds of spontaneous fetal aberrations in the control population of various laboratory species are often very high. A large proportion of these are readily identified during routine fetal examination. Others, such as alterations in the course of blood vessels, are inconspicuous and liable to go unidentified. The role of almost all the aberrations in normal development is speculative and factors governing their expression are elusive. A number of these have been shown to increase both in frequency and morphologic expression, following maternal treatment with a variety of chemicals. In teratologic assessments, the precise significance of this increase in extrapolation of human risk is not known but is urgently needed.

The purpose of this paper is to review the incidence, in control and drug-treated groups, of some of the more commonly occurring fetal aberrations and to discuss their teratologic significance. Retarded ossification of sternum, hydronephrosis and undescended testis, are selected as examples of the fetal retardation, while supernumerary rib and wavy rib represent, respectively, fetal variation and fetal deviation.

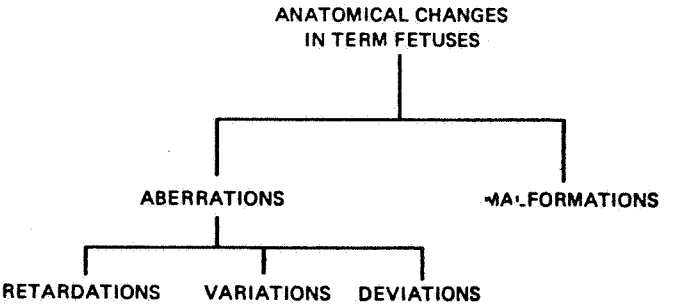

FIG. 1. Types of anatomical changes.

*Presented at the Third International Caffeine Workshop, Hunt Valley, Maryland, October 26-28, 1980.

FETAL RETARDATION

Retarded ossification of sternum and sternal defects

The degree of skeletal ossification in the fetus has generally been recognized as an indicator of growth and development. During prenatal development, skeletal ossification may be retarded by test materials that alter maternal homeostasis. Following maternal treatment, large numbers of chemicals are known to decrease ossification in fetal calvarium, vertebral bodies, sternum, phalanges or calcaneus. Ossification in the sternum, for unknown reasons, is most readily inhibited during the last 2-3 days of pregnancy, coincidental with the removal of the fetuses for examination.

Normal sternal development

The prenatal development of the sternum has been described in detail for several vertebrate species (Gladstone and Wakeley, 1932) including man, pig, and cat (Whitehead and Waddell, 1911; Hanson, 1919) and mouse (Bryson, 1945; Chen, 1952 and 1953, Globus and Gibson, 1968). The following is a brief summary of the sternal development of the rat and mouse compiled from some of these sources.

Sternal development begins with the appearance of two widely separated bands, one on either side of the dorso-lateral body-wall, ventral to and sharply defined from the ribs. The two primordia lengthen in a caudal direction and more towards the mid-line with the down-growing of the tissues along the dorso-lateral body-wall. By the 16th day *in utero*, the primordia fuse anteriorly at the level of the first sternal rib of the rat and then fusion proceeds posteriorly so that, by the 18th day of gestation, a single bar of cartilage results. The rib-tips subsequently come in contact with the sternum. On the 20th day, hypertrophy in the sternal cartilage begins, which subdivides the sternum into sternebrae. Hypertrophy is followed by ossification which occurs in six linearly arranged centers. Thus at birth the sternum exhibits six well ossified segments interconnected by bands of cartilage at the sites of future sternocostal articulations. The ossification centers of the sternum in all mammals including man, bear a constant relation to the position of the ribs.

A study of gross morphology of the sternum in near-term alizarin-stained rat fetuses and postnatal pups from two different strains, has revealed interesting features. The number of ossification centers in untreated Wistar rat fetuses on the 19th, 20th, and 21st day of gestation (positive vaginal smear on day 0 of gestation) was quite variable. More than 90% of the fetuses had no or only one ossification center on the 19th day of gestation; nearly all fetuses had 4-6 sternebrae on the 20th day of gestation and almost all fetuses had six ossified sternebrae on the 21st day of gestation (Aliverti *et al.*, 1979). For untreated Sprague-Dawley rats, sternal ossification centers were detected in 90% of the fetuses taken 2 days before term; the fifth sternebra was represented by two separated ossification centers. Two days after parturition, the sternebrae were completely ossified in all neonates. An additional ossification center between the 5th and 6th sternebrae was noticed in 2% of the term fetuses, 3% of the neonates at 2 days of age and 12% of the neonates at 12 days of age (Fritz and Hess, 1970).

Sternal defects and retarded sternal ossification

Congenital defects of sternal ossification do not appear to be common in humans. In a survey of the offspring of 50 282 women, clinical examinations revealed defective sternums in two children (hypoplastic sternum in one and bifid sternum in the other (Heinonen *et al.*, 1977). Other congenital malformations in man are: fused sternebrae (Dwight, 1890), irregular ossification and non-segmentation of the sternum (Warkany and Nelson, 1941), bifid sternum (Paterson, 1900), cleft sternum or sternoschisis with or without ectopia cordis (Greig, 1926), pectus excavatum (Brown, 1939), pectus perinatum (Lester, 1953) and other minor malformations. The minor malformations include unapposed or malaligned sternebrae (resulting in assymetrical sternocostal articulations), 'scrambled' or disorganized sternebrae and crowding of ribs (resulting in an altered number of sternocostal articulations).

Initial investigations with thalidomide failed to reveal any significant effect on sternal development in rats. But later, in an effort to show that rats were also responsive to thalidomide, workers reported that retarded ossification of the sternum was observed in fetuses from rats treated with high doses of thalidomide. In those studies (Klein, Obbink and Dalderup, 1963; Dwornik and Moore, 1965; Cook and Moore, 1967; Jonsson, 1972), the fetuses were collected one day before term and the etiologic role of other factors (including reduction in fetal weight) was not discounted as being responsible for retarded sternal ossification. Although a number of subsequent studies in the rat have not revealed a direct cause and effect relationship between thalidomide and retarded ossification of the sternum (Werth and Hirth, 1968; Fratta, 1969; Scott *et al.*, 1978), the undue teratologic significance initially attached to retarded sternal ossification still continues but should be discouraged.

Retarded sternal ossification has been observed in a large number of studies. It occurred in the absence of significant fetal weight reduction following the administration of acetylsalicylic acid or phenobarbital to pregnant rabbits (McColl *et al.*, 1967). In some cases it appeared to have been related to the reduction in mean fetal body weight, *e.g.* dimethadione (Buttar *et al.*, 1978) and halothane (Lansdown *et al.*, 1976b). In other cases it occurred sporadically regardless of the dosages tested: isoniazid and ethionamide (Dluzniewski and Gastol-Lewinska, 1971), malathion and methoxychlor (Khera *et al.*, 1978), piperonyl butoxide and phosalone (Khera *et al.*, 1979a) and lindane (Khera *et al.*, 1979b). However, there is some evidence to indicate that the mechanism of retarded ossification may be more complex than the preceding studies suggest. For example, maternal protein deprivation significantly retards the development of ossification centers in the rat. A linear relationship apparently exists between fetal body weight and the number and shape of ossification centers, such that the greater the weight of the fetus, the greater the number of ossification centers present (Schrader and Zeman, 1973). A strong inverse and linear relationship between litter size and the number of ossification centers in the sternum, such that the larger the litter size, the smaller the number of ossification centers in the rat has also been reported (Knight and Ree, 1978). However, litter size has also been inversely related to fetal body weight (Marthens and Grauel, 1974). Therefore it is probable that the effect of litter size on ossification of sternum may be a secondary effect which is largely due to delayed fetal maturation in the smaller fetuses. It is pointed out that the number of ossified coccygeal vertebrae was also shown to be positively correlated with body weight of full term fetuses in control groups of ICR-JCL mice and Wistar rats. A similar correlation was also obtained for mice treated with thio-TEPA and for Wistar rats treated with 4-chloro-2-methylphenoxyacetic acid ethylester (Ariyuki *et al.*, 1980).

The variability in sternal ossification in 19-day old (2-day preterm) fetal rats becomes more uniform with the approach of parturition. It is suggested that live fetuses for examination should therefore be removed on the last day of gestation (*i.e.* on day 21 if the day of finding sperm in the vaginal smear is as being counted day 0 of pregnancy) and the state of ossification in runts is scored separately (Table 1).

The retardation of sternal ossification could occur as a result of decreased maternal body weight gains during pregnancy, a reduction in fetal body weight, or secondary fetal immaturity due to a large litter. Contributory roles of these factors should be clearly discounted before relating the retarded ossification directly to the test compound. Retarded sternal ossification, however, could occur in the absence of any of the changes outlined above. A postnatal study of the skeleton is then suggested to test the potentiality of the sternum to develop into its normal shape and size and to determine the additional time needed by the affected sternum to attain the control size.

Hydronephrosis

Hydronephrosis is characterized by distension with urine of the pelvis and calyces of the kidney (as a result of obstruction in the ureter) and accompanying atrophy of the renal cortex. The diagnosis of fetal hydronephrosis is at present based on the degree of dilatation of the renal pelvis, reduction of the renal papilla and reduction of the renal cortex. These criteria do not seem to be valid, since the dividing line between physiological and pathological variations of these structures in the fetus, have never been defined. In the kidneys of control fetal rats 4-5 days before parturition, a markedly higher growth rate in the parenchyma than in the papilla has been found to cause the formation of a large pelvic cavity which was indistinguishable from that of a genuine hydronephrotic kidney (Woo and Hoar, 1972). In rat fetuses from dams treated with methyl salicylate, the true nature of the enlarged pelvic cavity (physiologic or hydronephrotic, and transitory or permanent) became apparent only after the kidneys from pups reared until weaning, were examined (Woo and Hoar, 1972). An enlarged or dilated pelvic cavity, accompanied by either dilated and tortuous ureters, or ureters terminating in a blind pouch, could be suggestive of hydronephrosis caused by urinary stasis. However, when found alone, the diagnosis should be based upon a study of weanlings.

Various morphologic and functional abnormalities of the kidney that occur during fetal development have been reviewed (Gibson, 1976; Monie, 1977). Fetal hydronephrosis can occur from mechanical impediment in the urinary flow (generally in the vesico-ureteral (Bagg, 1929) or uretero-pelvic regions) or in the absence of any overt obstruction. Hydronephrosis has been produced in the offspring of rats kept on diets deficient in pteroylglutamic acid (Monie *et al.*, 1954) or pantothenic acid (Roux and Dupuis, 1961) or in rats treated with large doses of vitamin A (Giroud *et al.*, 1959). X-irradiation of mouse embryos on 9 1/2 - 10 1/2 days of gestation was also found effective (Russell, 1950).

Undescended testis or slow descent of testis

Descended testis denotes the transference of the testis from the abdomen to the scrotum. During early embryonic development, the testes are attached to the dorsal abdominal wall by urogenital mesenteries. In the fetal rat at term, the testis and epididymis are found in the pelvis beside the bladder near the inguinal fossa. After birth the testes, aided by the gubernaculum testis, pass through the inguinal canal thereby invaginating the processus vaginalis, and reach the scrotal sac. The testicular descent is under hormonal control. Administration of gonadotropins and androgens can induce testicular descent in cryptorchids. Atrophy of Leydig cells caused by estrogen given on day 14 of pregnancy has been incriminated in causing cryptorchidism (Hadziselimovic and Girard, 1977). Leydig cells act as target tissue for estrogen because they have estrogen-binding protein receptors, and they respond by secreting less of the enzyme that converts progesterone to testosterone (Huseby, 1976). Besides hormonal deficiencies, failure to descend has been attributed to abnormalities in the testis, vas deferens and gubernaculum, and to inguinal hernia.

In a teratologic study on term fetuses, fetal testes found part-way between the kidney and inguinal fossa may represent either a transitorily retarded descent quite likely to be compensated for during postnatal growth, and therefore a minor effect, or a more serious consequence of cryptorchidism. In the absence of a postnatal study, it is inappropriate to hazard a guess on the significance of this finding.

An inhibitory effect on the descent of fetal testes, which were found lying part-way between the kidney and the inguinal fossa, has been assigned to a number of chemicals: Win 18 446 (N,N'-bis-(dichloroacetyl)-1,8-octa-methylene diamine (Talepros *et al.*, 1978), cyclophosphamide (Singh and Raju, 1974) and chlorcyclizine (Auerbach and Barrow, 1972) in the rat and DDVP or dichlorvos in the mouse (D'Souza and Batra, 1976).

FETAL VARIATION

Supernumerary rib

(Extra rib, cervical or lumbar rib, complementary rib)

Supernumerary ribs in man may be found associated with the last cervical or the first lumber vertebra. The presence of a lumbar rib was identified in 17 individuals following the examination of 38 105 roentgenograms. Twelve of the 17 individuals with a lumbar rib had moderate to severe backache (Steiner, 1943). An estimate of 2% has been suggested for all rib anomalies (including supernumerary ribs) in man (Coury and Delaport, 1963). The estimated incidence of cervical rib in man was 0.03 to 0.1% (Murphy, 1916) and seemed to be genetically determined, being commoner in the female (Davis and King, 1938). The cervical rib was asymptomatic in 50-75% of cases, but in others it interfered in the functioning of the brachial plexus or the subclavian artery by mechanical pressure (Adson and Coffee, 1927). Cervical ribs are normal in crocodiles (Eisendrath, 1904), but in mammals their presence

TABLE 1

Factors for Assessment of Retarded Sternal Ossification

1. Fetuses to be delivered on last day of pregnancy
2. Runts to be scored separately.
3. Contributory role of the following to be clarified:
   (i) Changes in maternal body weight during pregnancy.
   (ii) Reduction in mean fetal weight.
   (iii) Large litter size.
   (iv) Generalized inhibited ossification in fetal skeleton.
4. If the retardation is specific, a postnatal study on sternum is indicated

has been viewed with some degree of skepticism. The occurrence of cervical and lumbar ribs is highly variable dependent upon the species and strain of mammal. There are no data showing that, in the laboratory rodent, it is incompatible with life.

The spontaneous occurrence of supernumerary ribs in normal rabbits has been shown to be highly variable and determined by both genetic and extragenetic factors which may influence the differentiation and growth of rib precursors at a specific stage of embryogenesis. It may also be transmitted alone or, less commonly, with an extra pre-sacral vertebra, ventral spinous process and sternebra (Green, 1939).

The supernumerary rib may vary in size from rudimentary to that of a normal rib (Cozen, 1965). It has been observed in untreated or vehicle-treated mice, hamsters, rats, rabbits, cats and dogs. In vehicle-treated animals an incidence rate of 25% in ICR-JCL mice (Imahori, 1975), 40% in C_{57} BL mice (Fuyuta *et al.*, 1978), 21% in golden hamsters (Beatty and Hillemann, 1950; Gale, 1975), and 36% (Yasuda and Maeda, 1973) in Sprague-Dawley rats, is known. The occurrence of supplementary ribs in rats did not appear to be sex-linked (Lansdown, 1976). In the rabbit, an incidence of 12.4% (Palmer, 1968) and 1.5% (Fritz, 1975) has been reported. The rib counts are apparently variable in rabbits and usually constant for a given strain in the mice and rats. The incidence in fetal dogs (Robertson *et al.*, 1979) and cats (Khera *et al.*, 1976b) appears to be low, based upon relatively small sample sizes.

Chemical agents may significantly increase the incidence of extra rib in fetuses with overt malformations. Ytterbium chloride increased the incidence of supernumerary ribs to 68% in golden hamsters (Gale, 1975), and dimethadione to 95% in Wistar rats (Buttar *et al.*, 1978). Drug induced increases in the frequency of extra ribs may prove to be species-specific. An increase was observed following treatment with acetylsalicylic acid in rats (Kimmel *et al.*, 1971; Tanaka *et al.*, 1973) but not in cats (Khera, 1976a) or dogs (Robertson *et al.*, 1979). Vitamin A increased the incidence in Sprague-Dawley rats (Yasuda and Maeda, 1973) but not in dd N and CF #1 strains of mice (Murakami and Kameyama, 1966). Phenobarbital sodium markedly increased the incidence in Syrian hamsters in our laboratory but had no effect in Sprague-Dawley rats (McColl *et al.*, 1963) or mice (Sullivan and McElhatton, 1975). Phenobarbital treatment combined with an atmosphere of low oxygen concentration did however increase the incidence of 14th ribs in Sprague-Dawley rats (Mackler *et al.*, 1975).

The incidence of 14th rib is increased in a dose-dependent manner by a large number of chemical agents that produce no overt malformations. The tetracycline derivatives (Yasuda and Maeda, 1973) and metiazinic acid (Nakamura *et al.*, 1974) in mice, pentachlorobenzene in rats (Khera and Villeneuve, 1975), and photomirex in rabbits (Villeneuve *et al.*, 1979), are a few examples. Extra ribs have been interpreted by some to be an indicator of teratogenicity (Yasuda and Maeda, 1973). Maternal stress and frank embryo-toxicity attributed to maternal treatment at high doses have been associated with the extra ribs reported by others (Kimmel and Wilson, 1973). Studies in which the dose-related extra rib was the only positive finding, have been variously interpreted. The finding was considered to be of no significance by some (Khera, 1974; Hudak and Unguary, 1978) or as being suggestive of teratogenic activity by others (Basford and Fink, 1968).

FETAL DEVIATION

Wavy rib

(Undulating rib, bent rib)

The 'wavy' or 'bent' rib is a collective name for congenital undulations in several ribs arranged in a characteristic pattern. It generally occurs bilaterally and the incidence in control Wistar rat fetuses is 2.7% (Khera, 1970).

Wavy rib has been produced in mice and rats by a number of chemicals examined for teratogenic activity including monolinuron and buturon (mice) (Matthiaschk and Roll, 1977) and methoxychlor (rats) (Khera *et al.*, 1978). This deviation may be species-specific since methylmercuric chloride produced wavy ribs in Wistar rats but not in C57 BL mice (Fuyuta *et al.*, 1978). Netilmicin (Sch 20569), a semi-synthetic aminoglycoside antibiotic, was associated with this anomaly in CD rats but not in rabbits (Bamonte *et al.*, 1979). Ochratoxin A caused wavy ribs in mice (Hayes *et al.*, 1974) and Sprague-Dawley rats (Brown *et al.*, 1976) but not in hamsters (Hood *et al.*, 1976).

The initial lesion in the rib was noticed in 18-day-old rat fetuses as an extensive area that was markedly deficient in calcium deposition (and also alkaline phosphate activity). The movement of cervical and abdominal muscles with costal attachments and fetal movements, could create forces pulling in opposite directions. Under this muscular stress, incompletely ossified ribs could respond by assuming an undulated or wavy appearance (Khera, 1970). β-Aminopropionitrile has been shown to produce a 100% incidence of undulating rib and a defect in the extracellular maturation of collagen has been reported as the underlying cause (Wiley and Joneja, 1978).

CONCLUSIONS

Fetal aberrations occur quite commonly in tests conducted to determine the teratological potential of chemicals. Their incidence and morphology are highly variable and the significance far from clear. If they are present in conjunction with frank malformations, the latter would obviously provide the basis for extrapolation to human safety. When aberrations occur alone in a test, the findings are hard to interpret, because appropriate information on their significance is not available. At times, their markedly elevated incidence may suggest something of a pathological significance, but on other occasions their similarity to the incidence and distribution in controls, may not warrant serious attention.

For a more precise extrapolation of animal studies to the evaluating of human safety, the cause and significance of fetal aberrations must be established before teratologic studies can be more precisely interpreted. Specially designed studies which are scientifically sound must be executed to produce results that help establish guidelines for the extrapolation of animal data to human safety. A method based upon postnatal assessment of skeletal variations of mice has recently been proposed to detect prenatal exposures to harmful chemicals (Beck, 1981).

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