

Anilin

(CAS-NR.: 62-53-3)

A) Genotoxicity:

In vitro investigations:

Studies in bacteria:

Studies evaluating the genotoxicity of aniline and other splenic carcinogens have shown negative or inconclusive results in standard Ames test protocols including *Salmonella typhimurium* strain TA 102 (Jung et al., 1992; Rosenkranz and Poirier, 1979; Wilmer et al., 1981; Haworth et al., 1983; Bus and Popp, 1987; Simmon, 1979; DeFlora, 1981; Dunkel et al., 1984/85; Nakamura et al., 1987; Sakagami et al., 1988; Shahin, 1989).

In the presence of norharman, mutations in strain TA 98 were observed after metabolic activation (Nagao et al., 1977; Sugimura and Nagao, 1981). The norharman modification of the Ames assay, however, is based on a chemical reaction product between aniline and norharman in the incubation medium (aminophenylnorharman and hydroxyaminophenylnorharman; Totsuka et al., 1998) and therefore artificial in nature.

A positive result was also reported from preincubation with human gastric juice (DeFlora et al., 1980/82).

A metabolite of aniline, phenylhydroxylamine, was shown to be mutagenic in *Salmonella typhimurium* strain TA 100 when metabolically activated with S-9 mix preparation (Nohmi et al., 1984).

Urine samples (ether extracts) of rats receiving 300 mg aniline p.o. 24 hours before sampling showed mutagenicity in TA 98 in the presence of S-9 mix (Tanaka et al., 1980).

In *Bacillus subtilis* recombination assays positive (Schiestl et al., 1989) and negative results (Mamber et al., 1983; McCarrol et al., 1981; Simmon et al., 1979) were obtained. DNA repair assay in *E. coli* Pol A⁺/A⁻ was negative (Fluck et al., 1976; DeFlora et al., 1984; Rosenkranz et al., 1984), also DNA fragmentation (Rosenkranz et al., 1984).

Studies in mammalian cells:

UDS tests on hepatocytes of rats, mice, hamsters and humans showed negative results after autoradiographic evaluation; concentrations up to 1.0 mmol (93 µg/ml) were employed (Williams, 1980; McQueen et al., 1981; Yoshimi et al., 1988; Butterworth et al., 1989). In one assay employing a non-autoradiographic evaluation via BrdU incorporation, subsequent density shift gradient separation and liquid scintillation counting a positive result was generated (Andrae, 1986).

In an HGPRT assay on V79 cells Aniline exerted a weak activity at extreme concentrations (4,600 and 5,600 µg/ml) in the presence of metabolic activation (Fassina et al., 1990).

In mouse lymphoma cells, Aniline showed effects in the TK⁺ assay thymidine kinase locus with and without metabolic activation. Effective concentrations were in the range of 46.5 – 465 µg/ml with S-9 mix and 930 – 1,350 µg/ml without S-9 mix (Wangenheim and Bolesfoldi, 1988; McGregor et al., 1991; Mitchell et al., 1988). Myhr and Caspari (1988) found only weak effects at high concentrations, whereas Amacher et al. (1980) found a negative result (up to 1,100 µg/ml). Differentiations between large and small colonies were generally not recorded; therefore the effects may account for chromosome aberrations.

In mouse lymphoma cells and the presence of S-9 mix, a DNA fragmentation was obtained in a concentration of 21.5 mmol/l which is unusually high (ca. 2,400 µg/ml; Garberg et al., 1988).

No DNA fragmentation was found in V79 cells (Swenberg, 1976/81) or in human fibroblasts (Kozumbo, 1992).

Chromosome aberration assays in various hamster cell lines showed several positive results (Galloway et al., 1987: 1,600 and 5,000 µg/ml; Miltenburger et al., 1986: 4,300 µg/ml; Ishidate et al., 1988: 1,000 and 2,000 µg), that were typically obtained at high concentrations; other authors found negative results (up to 10⁻² M; 1,400 µg/ml of aniline-HCl; Abe and Sasaki, 1977; Swenberg et al., 1976).

Weakly increased SCE rates were observed in hamster cells (Abe and Sasaki, 1977; Galloway et al., 1987) and a rat liver cell line (Cunningham and Ringrose, 1983). For the study of Abe and Sasaki (loc. cit.) the unusual length of incubation period (26 hours) has to be pointed out, which may have facilitated a spontaneous oxidation of the test material; furthermore, no dose-response relation was apparent. Galloway et al. found 3,000 – 5,000 µg/ml as effective concentrations (with S-9 mix).

In human fibroblasts (465 and 930 µg/ml; Wilmer et al., 1981) or concanavalin A-induced human lymphocytes (up to 93 µg/ml; Wilmer et al., 1984), increased SCE rates were recorded with aniline HCl. The authors also showed that this effect does not occur with purified lymphocytes and that the addition of hemoglobin (1,000 µg/ml) facilitates the expression of SCEs. The metabolites o-aminophenol and N-phenylhydroxylamine were more potent SCE inducers than aniline itself and produced a dose-dependent increase in SCE rates in human fibroblasts (0.05 and 0.1 mmol/l; Wilmer et al., 1981). Other authors (Tohda et al., 1983; Takehisa and Kanaya) showed increased SCE rates after norharman addition which is an artificial modification according to today's knowledge (see above: studies in bacteria).

According to a poorly documented investigation, aniline did not produce micro-nuclei in SHE cells (Fritzenschaf et al., 1993).

In vitro cell transformation assays in SHE and BHK21 cells were negative (Dunkel et al., 1981; Pienta et al., 1981; Rosenkranz et al., 1984; Amacher et al., 1982; Styles, 1980). In the BALB/3T3 cell transformation assay a positive result in the absence of S-9 mix was obtained (Dunkel et al., 1981; Rosenkranz et al., 1984).

In vivo investigations:

In mice, induction of micronuclei after single administration was limited to the top dose levels only (tab. 1a) which were close to LD₅₀ value and coincided with severe (hemato)-toxicity and a relative increase (in percentage) of polychromatic erythrocytes to normochromatic erythrocytes. Sometimes the micronuclei showed atypical morphology (Vlachos, 1989; Westmoreland and Gatehouse, 1991; Ashby et al., 1991). Negative results were obtained by Harper et al. (1984) and by BG (1985) at dose levels of 125 and 250 mg/kg and 610 mg/kg, respectively. More recently, a dose related increase of micronuclei rates was observed in B6C3F1 mice also after subchronic feeding with 500, 1,000 and 2,000 ppm (Witt et al., 2000).

The positive MNT-result was also confirmed with the acridine orange staining method (Ashby et al., loc. cit.), which is DNA-specific.

All dose levels that were employed in these studies are clearly hematotoxic. The possibility of micronuclei arising by a non-genotoxic mechanism has already been reported in the literature: bone marrow stimulation may enhance the formation of micronuclei, as was shown e.g. after administration of erythropoietin preparations or prostaglandin E₂ (Suzuki et al., 1989 and 1994) or after bleeding (Hirai et al., 1991). In all these cases the employed staining method was Giemsa, which may also cross-stain with Heinz bodies.

In order to further discern between a direct (genotoxic) or a possible indirect (hematotoxic and myeloproliferative) increase of micronuclei rates in mice after aniline administration, a bone marrow metaphase study was carried out which addresses the question on clastogenicity in a more specific way than the MNT (CTL, 2001a). In this study, groups of mice received two i.p. injections, separated by 24 hours, of each 220, 300 and 380 mg/kg aniline HCl; samples were taken after 16, 20 and 24 hours and 100 metaphases per animal were counted. No positive effects were observed in this study.

A number of micronucleus assays has been carried out also in rats (table 1a):

In an earlier experiment in PVG rats a dose-related increase of micronuclei rates was recorded at 400 and 500 mg/kg aniline HCl after 48 hours. After 24 hours increases were recorded at 287 and 400 mg/kg with an inverse dose relation and a nearly normal rate at 500 mg/kg (tab. 1b). The percentage of polychromatic erythrocytes was decreased after 24 hours and increased after 48 hours, thus reflecting bone marrow imbalances, presumably due to hematotoxicity (George et al., 1990).

Since the experiment of George et al. employed Meyer's haemalum and eosin as staining reagent, which is not DNA specific and may confound with other types of microbodies that do not represent DNA fragments such as e.g. Heinz bodies (resulting from MetHb formation) or mast cell granula on the slides (which are typical for rats, not mice), a recent reinvestigation was carried out in the same strain of rats yet employing a different and DNA-specific staining method (acridine orange). This time, a negative result was obtained after 48 hours and a dose-related increase in micronuclei rates after 24 hours at 300, 400 and 500 mg/kg (n = 7; tab. 1b; CTL, 2001b). In order to understand these contradictory results between the George et al., and the recent CTL experiment and in order to differentiate whether these observations stem from a true clastogenic effect or, again, from bone marrow

imbalances and hematotoxic properties, a metaphase study from rat bone marrow has to be carried out as was done before in mice.

DNA fragmentation (alkaline elution assay) was observed in kidney and liver but not in spleen of rats after single i.p. injection of 210 and 420 mg/kg (Parodi et al., 1982). The same group of authors did not find DNA-fragmentation in livers or bone marrow of male Swiss mice under the same treatment regimen; whereas in kidney the results were inconsistent (positive after 300 mg/kg i.p.; Cesarone et al., 1982 and negative after 420 mg/kg i.p.; Parodi et al., 1982). Repeated dosing was not investigated.

The same authors recorded also an increased SCE rate in the bone marrow of mice treated with 210 and 420 mg/kg i.p. (Parodi et al., 1982/1983).

A low range of DNA-labeling in kidney, liver and spleen was found within the frame of a preliminary experiment in rats 24 and 6 hours after an i.p. ^3H -aniline injection (Roberts and Warwick, 1966). A later conversion of these data into a CBI* value showed that if all label was due to covalent binding, a CBI of 3.7 as an upper limit for a putative DNA binding potency would result for liver (Lutz, 1979); if the study had been carried out properly and according to today's state of the art; however, neither purification steps to a constant specific activity of DNA nor a nucleotide analysis in order to discern between true binding, non-covalent association of radiolabel and metabolic incorporation has been performed (Baertsch et al., 1991; Lutz, 1993).

In a more recent study employing i.p. injection of aniline after a pretreatment phase (7 x 50 mg/kg + 1 x 250 mg/kg ^{14}C -aniline) rats, a preliminary "CBI" value of 14.2 for kidney was obtained. In naive rats treated only once, a "CBI" of 7.4 was recorded. For spleen and large intestine the calculated "CBI" values were between 0.5, 3.7 and 4.3, respectively. No radiolabel allowing "CBI" calculation could be obtained for liver (McCarthy et al., 1985).

In mice, spleen "CBI" values ranged from 1.0 to 1.5; no other substantial DNA binding was detected in mice (McCarthy et al., 1985). Also in these experiments, DNA was not purified to constant specific activity. The CBI levels therefore can only represent upper limit values. The sex of the animals was not reported, which is a deficiency since the spleen tumors are quite sex-specific.

Sasaki et al. (1999/2000) conducted alkaline single cell gel electrophoresis assays ("comet assays") in mice in samples from stomach, colon, liver, kidney, bladder, lung and bone marrow (- not spleen), 3 and 8 hours after single oral treatment with 1,000 mg/kg. The investigated organs had not been perfused but homogenised before isolating the nuclei; cytotoxic effects could therefore not be assessed, thus, positive effects are of doubtful toxicological significance and do not necessarily indicate a specific genotoxic DNA fragmentation.

A dominant lethal assay employing groups of 40 fertile male rats receiving 75, 150 or 200 mg/kg/day i.p. for 5 consecutive days and a subsequent 10 weeks mating period did not show effects in the two lower dose groups. In the highest dose 2 rats died showing toxic effects on day 4 and 5, the other rats showed clinical signs of toxicity at week 3 of mating. A slight decrease in the number of life implantations was found when compared to the concurrent control group; these findings appeared to focus in a subgroup of 7 animals with clearly reduced numbers of life implants and elevated

* CBI = Covalent binding index

numbers of dead early implants. The overall results were therefore classified as negative by the authors (CTL, 1998).

Furthermore, there was no increase in sperm head anomalies in mice 5 weeks after 5 daily injections of aniline HCl (17 – 200 mg/kg; Topham et al., 1980).

B) Carcinogenicity:

1. Carcinogenicity studies in rats, mice and hamsters:

Aniline – investigated as aniline hydrochloride (aniline-HCl) – was shown to induce malignant spleen tumors in rats in 2 long-term bioassays (NCI, 1978; CIIT, 1982). Mice were non-responsive (NCI, 1978). A limited experiment in hamsters did not show tumors either (Hecht et al., 1983).

An earlier study in which aniline-HCl was fed to Osborne-Mendel rats for 420 – 1032 days (330 ppm; n = 40 males and (?) 40 females) showed 3/43 sarcomas of the spleen, fibroses (14) and hemorrhages (23) of the spleen and 4/43 (benign?) hepatomas and possibly fibrosis (?) of the liver (White et al., 1948). This study presents only a limited information, since some important details such as the purity of the test substance and sex distribution of the tumors are missing. The liver findings were not confirmed in the later studies (see below).

Other earlier studies employing drinking water exposure of rats at daily doses of 22 mg/animal (about 100 mg/kg bw; n = 50; maximum exposure time 750 days; mean exposure time > 425 days; Druckrey et al., 1950) or in concentrations of 300, 600 and 1,200 ppm (n = 10 – 18; 80 weeks; Hagiwara, 1980) showed no tumors, but also these investigations appear to be compromised by insufficient documentation (e.g. of sex distribution and toxicity), limited numbers of animals or too short exposure times.

Syrian hamsters after a 1-year exposure time with weekly s.c. injections of 1.9 mmol/kg (177 mg/kg) of aniline (oil; total dose 99 mmol/kg; n = 30 males, 30 females) and a subsequent post-observation period of 35 weeks showed some indication of toxicity (decreased survival times) but not of tumorigenicity. Data on spleen toxicity and MetHb formation are missing. o-Toluidine at the same treatment scheme was also negative which indicates a low sensitivity of the test model employed (Hecht et al., 1983).

As the key studies for hazard assessment in rats and mice the long-term bio-assays of NCI and CIIT are generally employed. The tumor data of these studies are compiled in table 2.

In the CIIT study (CIIT, 1982; Bus and Popp, 1987) dietary concentrations of 200, 600 and 1,600 ppm (10, 30 and 100* mg/kg b.w. and day) were offered to 130 Fischer rats per sex and dose group:

* equivalent to 7, 22 and 72 mg free base

At 1,600 ppm typical hematotoxic effects with methemoglobinemia (3.63%), erythropenia, extramedullary hematopoiesis and spleen toxicity including fibrotic hyperplasia and capsulitis occurred in both sexes to a pronounced extent; however, with exception of capsulitis they were much more expressed in males than in females. Among the male animals of this group 34 spleen sarcomas of different histology were recorded; among the females only 1 animal had a spleen hemangiosarcoma. The spleen sarcomas were, besides general toxicity, a factor of premature deaths in this group. Increased liver weights occurred in both sexes.

At 600 ppm the number of premature deaths was not increased; 1 male animal showed a stromal sarcoma of the spleen. The spleens were darkened and enlarged; chronic capsulitis, however, was observed in only 2/128 examined males and 4/150 females. Hematotoxicity was still pronounced: reduced hemato-crit, Hb and RBC values were recorded, MetHb was 1.40% and increased hemato-poiesis was observable in bone marrow.

At 200 ppm there was still an increased hematopoiesis, but no marked spleen toxicity. 1 case of capsulitis was observed (just as in the control group). MetHb was 1.89%.

In the course of a preceding range-finding study 30 mg/kg/day (equivalent to 600 ppm) had caused increased MetHb and reticulocyte values. At 100 mg/kg darkened and enlarged spleens and discoloration of livers and kidneys were visible (Bus and Popp, 1987).

The NCI study (NCI, 1978) provided a diet of 3,000 and 6,000 ppm (aniline-HCl) to F344 rats for 103 weeks (175 and 360 mg/kg per day; n = 50; table 2b). In the high dose group 20/46 male animals showed hemangiosarcomas of the spleen; at other sites (mostly peritoneal cavity) 6 fibromas, 7 fibrosarcomas and 2 undefined sarcomas were also observed. The females at 6,000 ppm developed 7/50 benign and malign spleen tumors of different origin. At 3,000 ppm the males developed 19/50 spleen hemangiosarcomas and 14 other benign and malign tumor types in the spleen, 2 fibrosarcomas spreading into the body cavity. Among the females of the low dose 1/50 showed a hemangiosarcoma in the spleen and 1/50 a fibro-sarcoma in the body cavity.

Hemangiosarcomas and fibrosarcomas were observed also at other locations than spleen, since the fibrosarcomas showed histologically a high degree of malignity and infiltration into the peritoneal tissues. No indication of non-neoplastic alterations outside of the spleen was observed, though discrete cellular damages, e.g. of endothelial cells from circulating excess iron should not generally be excluded regarding the causes of some tumors at other sites than spleen.

The NCI report does not mention hematotoxicity or spleen toxicity. However, such effects were demonstrated in a preceding 8-week study at dietary levels of 300 ppm and higher; no effects were seen at 100 ppm (NCI, 1978) and in the course of a reinvestigation of this study by Goodman et al., 1984. Other authors found similar patterns with fatty morphosis, fibrosis, capsule hyperplasia and hemorrhage at 3,000 and 6,000 ppm (Weinberger et al., 1985; further investigations on the mechanism of aniline-related spleen toxicity are discussed below).

B6C3F1 mice were also investigated for carcinogenicity of aniline-HCl and showed negative responses in both treatment groups (6,000 and 12,000 ppm; 103 weeks; NCI, 1978). Survival rates were not affected. No increase of tumor incidences was observed. Again, hematotoxicity or spleen toxicity were not reported, but spleen toxicity in mice had been shown in a preceding 8-week feeding study, though at higher levels than in rats (NOAEL 45 mg/kg/day; LOAEL 450 mg/kg/day).

The relative carcinogenic potency of aniline HCl in the male Fisher rat was estimated in terms of TD₅₀ to 160 mg/kg/day in rats and > 9,000 mg/kg/day in mice (Gold & Zeiger, 1997).

2. Considerations on the mechanism of aniline-related spleen tumors in male rats:

Reactive metabolites like phenylhydroxylamine and nitrosobenzene (Khan et al., 2000) can be formed from aniline in the liver but and probably also in other organs with similar enzyme activities (cytochrom P450; N-aniline-hydroxylase). These metabolites are the chemical species considered to be responsible for the formation of hemoglobin adducts and the induction of hematotoxicity.

The spleen toxicity is explained by the fact that this organ stores an extremely large number of different types of blood cells and serves for the harvest of aged, decaying and damaged RBCs. At a high degree of hematotoxicity the spleen is loaded with cellular debris including also iron. The endothelial cells of spleen sinuses are ultimately overwhelmed by an excess of iron leaking from decayed RBCs. If these target cells are overwhelmed with iron exceeding their own iron binding capacities, iron-mediated Fenton reactions will occur and produce hydroxy radicals, DNA damage and other signs of oxidative stress (Khan et al., 1997). Activation of genes responsible for proliferation and fibroses drive the target cells towards malignant degeneration. Thus, aniline-related spleen toxicity is ultimately to be regarded as an epiphenomenon of hematotoxicity and Hb adduct formation.

Sex difference in susceptibility towards iron-related carcinogenicity has already been described with other chemicals and shows a much higher vulnerability of the male rat (Deguchi et al., 1995).

Interestingly, MetHb formation in plasma does not appear to be a parameter with a good prognostic value. This has been shown by the CIIT data but also by other authors (Neumann, 1988; Birner and Neumann, 1988; Neumann et al., 1991).

A very recent feeding study in male rats employed 3 increasing dose levels in the diet (10, 30, 100 mg/kg b.w./day) for 7 and 28 days. No elevation of MetHb levels was recorded at the time points of blood sampling (around 10.00 in the morning), but increases of Heinz bodies and hemoglobin adducts in a dose-dependent manner throughout all dose levels. RBC was slightly and numerically decreased at 10 mg/kg and significantly in a dose-related manner at 30 and 100 mg/kg. The top dose, which had earlier been shown to be carcinogenic to male rats, was – in addition – characterized by an increase of transferrin and iron binding capacity which mirror the perturbation of iron metabolism. Furthermore, the top dose specifically showed

significantly increased spleen weights after 28 days and a pronounced spleen pericapsulitis coinciding with an increase in WBC (BASF, 2001).

It appears therefore that, along the dose-effect relation, two separate dose ranges may be identified: a low dose range limited to hematological effects only and below the potential to cause spleen toxicity; and a higher dose range which would be prone to such damage and shows additional hematological effects. Thus, that hematological parameters from blood samplings can be employed to discern between these two dose ranges. Furthermore, the results support the view that aniline related tumors need a certain level of blood toxicity and the presence of spleen pericapsulitis. Fibrotic events were not visible at this early stage (28 days). Thus, the tumorigenicity does not appear to be driven by mutagenic effects of the test substance.

C) Toxicity for reproduction:

Developmental toxicity:

Pregnant Fischer 344 rats (n = 20) were administered via gavage 10, 30 and 100 mg aniline HCl/kg and day from gestation day 7 – 20. Hydroxyurea (200 mg/kg/d) was used as a positive control.

Dams of the top dose showed a statistically significant decrease (27.5%) in body weight gain and typical signs of aniline-related hematotoxicity (13.7% MetHb, decreased RBC and increased reticulocyte counts). A dose-related significant increase in maternal spleen weights was observed in all dose groups. Pregnancy rates, numbers of corpora lutea per dam, numbers of implantation sites and live fetuses per litter, fetal body weights and the fetal spleen weights were not affected. A 5% increase in fetal relative liver weights was recorded in the top dose. The incidences of malformations in all experimental groups including the control were: 3/178 in the vehicle control and 0/181, 7/210 and 4/190 in the 10, 30 and 100 mg/kg/day dose groups. Hydroxyurea produced 29.9 + 5.5% malformed fetuses per litter. Numbers and types of variations were not increased in the aniline-treated groups either. Thus, the study results are not indicative of a selective fetal toxicity.

A satellite group of animals was allowed to litter after treatment through parturition; the offspring pups were raised until day 60 p.p. The pups of the top dose group had reduced body weights. Dams of all dose groups showed increased spleen weights, increased MetHb levels and increased MCV on p.n. day 30. Offspring rats that were raised up to p.n. day 60 also showed occasionally increased liver weights and a slightly (not statistically significant) increased preweaning mortality, however, without signs of a deterioration of physical or behavioral development (Price et al., 1985).

In a Chernoff-Kavlock assay, 560 mg aniline/kg bw/d were administered by gavage to CD mice (n = 50) from gestational days 6 – 13. The maternal body weights were significantly reduced (9.1 → 6.7 g) and 6 out of 50 dams died during the treatment. (The dose had been predicted as LD₁₀.) There were no effects on the number of viable litters and peri/postnatal survival; the birthweights were somewhat lower

(1.6 → 1.5 g) than in the control and also the postnatal weight gain (1.1 → 0.9 g) during the first 3 days p.p. (Hardin et al., 1987). When the results are compared to those of compounds with clear developmental activity in the same study and the maternal toxicity is taken into account, the effects observed do not indicate a selective developmental toxicity of aniline in mice.

Effects on fertility:

At present no specific investigations are available. The subchronic and chronic toxicity studies that have been undertaken with aniline do not indicate testicular toxicity. Absolute and relative increases in ovary weights after 26, 52 and 78 weeks of feeding and an increased incidence of endometrial polyps at week 78 as well as decreased ovary weights at week 104 were recorded in the course of two long-term bioassay at hematotoxic dose levels bioassays; these may be spurious findings but were on the other hand relatively consistent between the two studies (CIIT, 1982; NCI, 1978). Aniline did not induce spermhead anomalies in mice (Topham, 1980 a + b).

D) Facit:

Mutagenicity:

In vitro data in bacteria and mammalian cell lines are either negative or equivocal and do not indicate gene mutations. Positive results in bacterial assays were obtained after incubation with human gastric juice or urine from aniline-treated rats.

Under high concentrations (> 1,000 µg/ml) and probably related to cytotoxicity, a clastogenic activity may be observed in certain cell lines, preferentially mouse lymphoma cells which have a low relation between cytoplasm volume and nucleus. Whether aniline or anilin HCl was employed, is not always recorded throughout the studies.

In vivo, aniline administration shows a propensity to produce increases in micronuclei rates in mice and rats at haematotoxic dose levels. A subsequent bone marrow metaphase study in mice with a negative result showed that in mice increased micronuclei rates presumably stem from the bone marrow stimulation as sequel of the hematotoxic effect rather than from a true clastogenic effect. In rats, such a study still has to be done.

DNA fragmentation in rat liver and kidney and increased SCE rates in the bone marrow of mice were observed, but do not necessarily indicate a propensity for clastogenic effects in these organs, as long as a discrimination to cytotoxicity has not been made. The available results do not to allow conclusions in terms of classification.

Dominant lethal and sperm head morphology tests were negative.

Reports on DNA binding in kidney and intestine after oral administration are based on improperly designed studies.

The increased micronuclei rates in rats in two somewhat contradictory experiments are so far unexplained and, on balance, suggest a classification into Muta. Cat. 3 (M: 3) as long as a negative rat bone marrow metaphase study has not shown that also in this species the increased micronuclei rates reflect bone marrow imbalances and hematotoxic properties of the chemical rather than a true clastogenic effect. If such a negative result can be obtained this classification may be revised.

The available study results do not indicate a primary genotoxic mechanism for the spleen tumors that were observed in rats after aniline administration.

Carcinogenicity:

Aniline is carcinogenic in rats, but not in mice. Male rats are far more sensitive than females.

Aniline is a typical hematotoxicant causing hemoglobin adducts and damage to erythrocytes. Hematological parameters including Heinz bodies, Hb adducts, RBC and WBC are suitable parameters to monitor exposure and the risk of persistent damage.

Decaying RBCs are harvested in the spleen and load this organ with excess iron. Oxidative stress and cytotoxicity will result from free iron after intercellular binding sites are overwhelmed (Khan et al, 1997; 1999). Thus, spleen toxicity which is a unique effect in chronic animal studies even with potent genotoxic carcinogens does not occur unless there is a preceding hematotoxicity. Moreover, at least in the case of aniline the spleen toxicity needs a high degree of severity in order to cause carcinogenic effects. Genotoxicity of aniline is not regarded as a constitutive element of the spleen tumors, which are non-genotoxic in origin and threshold related.

On balance, the following criteria support a classification into Carc. Cat 3 (C: 3) for aniline (in contrast to Carc. Cat. 2):

- Only one species (rat) is affected (males >> females).
- The target organ (spleen) is unique to hematotoxic chemicals.
- A steep dose response relation confines the tumors to high doses only.
- A high degree of long-lasting spleen toxicity is required for a tumorigenic effect.
- The spleen toxicity appears to be secondary to a preceding hematotoxicity which may be diagnosed by hematological parameters (and is to be prevented anyway).
- Aniline-related hematotoxicity leads to an iron overload in spleen resulting in oxidative stress, enforced cell proliferation, capsulitis and fibroses; if strongly pronounced, these effects are tumor-prone in rats; the higher susceptibility of the male rat is typical for an iron-mediated mechanism.
- No genotoxic mechanism for the spleen tumorigenicity in rats is assumed.

- The cytotoxic mechanism of the aniline-related spleen tumors is in its major aspects well elucidated and understood.

Developmental Toxicity:

Rats that were treated with aniline did not specifically show detrimental effects on the fetal and postnatal development in rats. In mice the picture is less complete since only a Chernoff-Kavlock assay has been carried out. However, the studies undertaken so far do not indicate a selective fetal toxicity or teratogenicity; classification of aniline is therefore not warranted (R_D,R_E: -).

Fertility:

Available studies do not clearly indicate selective effects on reproductive organs at hematotoxic dose levels. In the absence of more specific investigations concerning the endpoint fertility, a classification is at present not possible (R_F: -).

E. References:

- [1] Abe, S., Sasaki, M. (1977): Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals J. Natl. Cancer Inst., 58, 1635 - 1641
- [2] Amacher, D.E., Paillet, S.C., Turner, G.N., Ray, V.A., Salsburg, D. (1980): Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation Mutat. Res. 72, 447 - 474
- [3] Amlacher, E. (1982): Short-term tests in screening programs of environmental chemical carcinogens Exp. Path. 22, 187 – 202
- [4] Andrae, U. (1986): Aniline: - Test for the induction of DNA repair in rat hepatocyte primary cultures – Gesellschaft für Strahlen- und Umweltforschung Neuherberg
- [5] Ashby, J., Vlachos, D.A., Tinwell, H. (1991): Activity of aniline in the mouse bone marrow micronucleus assay Mutat. Res. 263, 115 - 117
- [6] Baertsch, A., Lutz, W.K. & Schlatter, C. (1991): Effect of inhalation exposure regimen in DNA binding potency of 1,2-dichloroethane in the rat. Arch. Toxicol. 65, 169 - 176
- [7] BASF AG (2001): Studie zum Wirkmechanismus an männlichen Fischer-Ratten. – Verabreichung im Futter bis zu 4 Wochen (99/298)
- [8] Beyerbach, A., Sabbioni, G. (1999): Biomonitoring of arylamines: Haemoglobin adducts of aniline derivatives Biomarkers, 4, 229 – 236

- [9] BG Chemie (1985): Mouse micronucleus test on aniline. Toxicol. Laboratories Ltd. Bromyard Road, Herefordshire, UK, sponsored by Berufs-genossenschaft der chemischen Industrie, Heidelberg, Germany
- [10] Birner, G., Neumann, H.-G. (1988): Biomonitoring of aromatic amines II: Hemoglobin binding of some monocyclic aromatic amines Arch. Toxicol. 62, 110 – 115
- [11] Bus, J.S., Popp, J.A. (1987): Perspectives on the mechanism of action of the splenic toxicity of aniline and structurally-related compounds. Fd. Chem. Toxicol. 25, 619 - 626
- [12] Butterworth, B.E., Smith-Oliver, T., Earle, L., Louny, D.J., White, R.D., Doolittle, D.J., Working, P.K., Cattley, R.C. Jirtle, R., Michalopoulos, G., Strom, S. (1989): Use of primary cultures of human hepatocytes in toxicology studies Cancer Res. 49, 1075 - 1084
- [13] Cesarone, C.F., Bolognesi, C., Santi, L. (1982): Evaluation of damage to DNA after in vivo exposure to different classes of chemicals Arch. Toxicol., Suppl. 5, 355 - 359
- [14] Chemical Industry Institute of Toxicology (CIIT) (1982): "Final Report, 104-Week Chronic Toxicity Study in Rats, Aniline Hydrochloride", Research Triangle Park, NC, USA
- [15] CTL (1998): Central Toxicology Laboratory, Aniline: Dominant Lethal Study in the rat, Report No. CTL/P/5404 Alderly Park Macclesfield, Cheshire, UK
- [16] CTL (2001a): Aniline hydrochloride: Mouse bone marrow metaphase test Proj. SM 1059
- [17] CTL (2001b): Aniline Hydrochloride: Rat bone marrow micronucleus test Proj. CO 0316r2; preliminary information to Bayer AG
- [18] Cunningham, M.L., Ringrose, P.S. (1983): Benzo(a)pyrene and aniline increase sister chromatid exchanges in cultured rat liver fibroblasts without addition of activating enzymes Toxicol. Lett. 16, 235 - 239
- [19] DeFlora, S., Boido, V., Picciotto, A. (1980): Metabolism of mutagens in the gastric environment Mutat. Res. 74, 187 – 188
- [20] DeFlora, S. (1981): Study of 106 organic and inorganic compounds in the Salmonella/microsome test Carcinogenesis 2, 283 - 298
- [21] DeFlora, S., Zancchi, P., Bennicelli, C., Picciotto, A., Boido, V (1982): Mutagenicity and interaction of chemicals in human gastric juice Mutat. Res. 97, 179 (Abstract No. 31)
- [22] DeFlora, S., Zancchi, P., Camoirano, A., Bennicelli, C., Badolati, G.S. (1984): Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test Mutat. Res. 133, 161 - 198
- [23] Deguchi, J., Miyamoto, M., Okada, S. (1995): Sex Hormone-dependent Renal Cell Carcinogenesis Induced by Ferric Nitrotriacetate in Wistar Rats Jpn. J. Cancer Res. 86, 1068 - 1071

- [24] Druckrey, H. (1950): Beiträge zur Pharmakologie cancerogener Substanzen – Versuche mit Anilin. Naunyn-Schmiedeberg's Arch. exper. Path. Pharmacol. 210, 137 - 158
- [25] Dunkel, V.C., Pienta, R.J., Sivak, A., Traul, K.A. (1981): Comparative neoplastic transformation responses of Balb/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical carcinogens J. Natl. Cancer Inst. 67, 1303 - 1315
- [26] Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S., Simmon, V.F. (1984): Reproducibility of microbial mutagenicity assays: I. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol Environ. Mutagen. 6, 1 - 254
- [27] Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S., Simmon, V.F. (1985): Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli* Environ. Mutagen. 7, 1 - 248
- [28] Fassina, G., Abbondandolo, A., Mariani, L., Taningher, M., Parodi, S. (1990): Mutagenicity in V79 cells does not correlate with carcinogenicity in small rodents for 12 aromatic amines J. Toxicol. Environ. Health, 29, 109 – 130
- [29] Fluck, E.R., Poirier, L.A., Ruelius, H.W. (1976): Evaluation of a DNA Polymerase-Deficient Mutant of *E. coli* for the Rapid Detection of Carcinogens Chem.-Biol. Interact. 15, 219 – 231
- [30] Fritzenschaf, H., Kohlpoth, M., Rusche, B., Schiffmann, D. (1993): Testing of known carcinogens and noncarcinogens in the Syrian hamster embryo (SHE) micronucleus test in vitro; correlations with in vivo micronucleus formation and cell transformation Mutat. Res. 319, 47 - 53
- [31] Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., Zeiger, E. (1987): Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ. Mol. Mutagen. 10, 1 - 175
- [32] Garberg, P., Åkerblom, E.-L., Bolcsfoldi (1988): Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution Mutat. Res. 203, 155 - 176
- [33] George, E., Andrews, M., Westmoreland, C. (1990): Effects of azobenzene and aniline in the rodent bone marrow micronucleus test Carcinogenesis 11, 1551 - 1556
- [34] Gold, L.S. & Zeiger, E. (1997): Handbook of Carcinogenic Potency and Genotoxicity Databases CRC Press, Boca Raton
- [35] Goodman, D.G., Ward, J.M., Reichardt, W.D. (1984): Splenic Fibrosis and Sarcomas in F344 Rats Fed Diets Containing Aniline Hydrochloride, p-Chloroaniline, Azobenzene, o-Toluidine, Hydrochloride, 4,4'-Sulfonyldianiline or D & C Red. No. 9 J. natl. Cancer Inst. 73, 265 - 273

- [36] Hagiwara, A., Masayuki, A., Hirose, M., Nakanowatari, I., Tsuda, H., Ito, N. (1980): Chronic effects of norharman in rats treated with aniline. *Toxicol. Lett.* 6, 71 - 75
- [37] Hardin, B.D., Schuler, R.L., Burg, J.R., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J. & Smith, K.N. (1987): Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratogen. Carcinogen. Mutagen.* 7, 29 - 48
- [38] Harper, B.L., Ramanujam, V.M.S., Gad-El-Karim, M.M., Legator, M.S. (1984): The influence of simple aromatics on benzene clastogenicity *Mutat. Res.* 128, 105 - 114
- [39] Haworth, S., Lawlor, T., Mortelmans, K., Speck and Zeiger, E. (1983): Salmonella mutagenicity test results for 250 chemicals *Environ. Mutagen. Suppl.* 1, 3 - 142
- [40] Hecht, S.S., El-Bayoumy, K., Rivenson, A., Fiala, E.S. (1983): Bioassay for carcinogenicity of 3,2'-dimethyl-4-nitrosobiphenyl, o-nitrosotoluene, nitrobenzene and the corresponding amines in Syrian golden hamsters *Cancer Lett.* 20, 349 - 454
- [41] Hirai, O., Miyamae, Y., Fujino, Y., Izumi, H., Miyamoto, A., Noguchi, H. (1991): Prior bleeding enhances the sensitivity of the in vivo micronucleus test *Mutat. Res.* 264, 109 - 114
- [42] Ishidate Jr., M., Harnois, M.C., Sofuni, T. (1988): A comparative analysis of data on the clastogenicity of 951 chemical substances in mammalian cell cultures *Mutat. Res.* 195, 151 - 213
- [43] Jung, R., Engelhardt, G., Herboldt, B., Jäckh, R., Müller, W. (1992): Collaborative study of mutagenicity with *Salmonella typhimurium* TA 102 *Mutat. Res.* 278, 265 - 270
- [44] Khan, M.F., Boor, P.J., Gu, Y., Alcock, N.W., Ansari, G.A.S. (1997): Oxidative Stress in the Splenotoxicity of Aniline *Fund. Appl. Toxicol.* 35, 22 - 30
- [45] Khan, M.F., Wu, X., Alcock, N.W., Boor, P.J., Ansari, G.A.S. (1999): Iron exacerbates aniline-associated splenic toxicity. *J. Toxicol. Environm. Health* 57, 173 - 184
- [46] Khan M.F., Wu, X., Ansari, G.A.S. (2000): Contribution of nitrosobenzene to splenic toxicity of aniline *J. Toxicol. Environm. Health* 60, 263 - 273
- [47] Kozumbo, W.J., Agarwal, S., Koren, H. (1992): Breakage and Binding of DNA by Reaction Products of Hypochlorous Acid with Aniline, 1-Naphthylamine, or 1-Naphthol *Toxicol. Appl. Pharmacol.* 115, 107 - 115
- [48] Lutz, W.K. (1979): In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis *Mutat. Res.* 65, 289 - 356
- [49] Lutz, W.K. (1993): Die Prüfung auf kovalente DNA Bindung. In *Mutationsforschung und genetische Toxikologie* (Fahrig, L., ed.), pp. 189 - 198. Wissenschaftliche Buchgesellschaft, Darmstadt

- [50] Mamber, S.W., Bryson, V., Katz, S.E. (1983): The Escherichia coli WP2/WP100 rec assay for detection of potential chemical carcinogens Mutat. Res. 119, 135 - 144
- [51] McCarrol, N.E., Keech, B.H., Piper, C.E. (1981): A Microsuspension Adaptation of the Bacillus subtilis "rec" Assay Environ. Mutagen. 3, 607 - 616
- [52] McCarthy, D.J., Waud, W.R., Struck, R.F., Hill, D.L. (1985): Disposition and metabolism of aniline in Fischer 344 rats and C57BL/6 x C3HF₁ mice Cancer Res. 45, 147 - 180
- [53] McGregor, D.B., Brown, A.G., Howgate, S., McBride, D., Riach, C., Caspary, W.J. (1991): Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals Environ. Mol. Mutagen. 17, 196 - 219
- [54] McQueen, C.A., Maslansky, C.J., Crescenzi, S.B., Williams, G.M. (1981): The genotoxicity of 4,4'-methylenebis-2-chloroaniline in rat, mouse, and hamster hepatocytes Toxicol. Appl. Pharmacol. 58, 231 - 235
- [55] Miltenburger, H.G. (1986): Test report of study LMP 102 (chromosomal aberration test in vitro for aniline), LMP, unpublished
- [56] Mitchell, A.D., Rudd, C.J., Caspary, W.J. (1988): Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Interlaboratory results for sixty-three coded chemicals tested at SRI international Environ. Molec. Mutag. 12, Suppl. 13, 37 - 101
- [57] Myhr, B.C., Caspary, W.J. (1988): Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Interlaboratory results for sixtythree coded chemicals tested at Litton Bionetics Inc. Environ. Molec. Mutag. 12 (Suppl. 13): 103 - 194
- [58] Nagao, M., Yahagi, T., Honda, M., Sieno, Y., Matsushima, T., Sugimura, T. (1977): Demonstration of mutagenicity of aniline and o-toluidine by norharman Proc. Japan Acad. 53, 34 - 37
- [59] Nakamura, S., Oda, Y., Shimada, T., Oki, I., Sugimoto, K. (1987): SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA 1535/psK 1002: examination with 151 chemicals Mutat. Res. 192, 239 - 246
- [60] National Cancer Institute (NCI; 1978): Bioassay of Aniline Hydrochloride for Possible Carcinogenicity" NCI-Bioassay Report No. 130, PB-287539, USDHEW, Bethesda, MD, USA; May 1978
- [61] Neumann, H.G. (1985): Appel, K.E., Hildebrand, A.G. (Hrsg.): "Tumorpromotoren. Erkennung, Wirkungs-mechanismen und Bedeutung" S. 98, Dokumentation eines Expertengespräches in Berlin am 6. und 7. Feb. 1984, bga Schriften 6/85, Max-von-Pettenkofer-Institut des Bundesgesundheitsamtes, Medizin Verlag München
- [62] Neumann, H.G. (1988): Haemoglobin binding in control of exposure to and risk assessment of aromatic amines. IARC Scientific Publications 89: Methods for detecting DNA damaging agents in humans: Applications in cancer epidemiology and prevention (Bartsch, H., Hemminki, K. & O'Neill, I.K., Eds.), International Agency for Research on Cancer, Lyon; pp. 157 - 165

- [63] Neumann, H.G., Birner, G., Sabbioni, G. (1991): Haemoglobin adducts of aromatic amines as a dosimeter for exposure control and risk assessment. in: Garner, Farmer, Steel, Wright (Eds.): Human Biomonitoring and Risk Assessment. IRL Press at Oxford University Press, pp. 337 – 344
- [64] Nohmi, T., Yoshikawa, K., Nakadate, M., Miyata, R., Ishidate, M. (1984): Mutations in *Salmonella typhimurium* and inactivation of *Bacillus subtilis* transforming DNA induced by phenylhydroxylamine derivatives *Mutat. Res.* 136, 159 - 168
- [65] Parodi, S., Pala, M. Russo, P., Zunino, A., Balbi, C., Albini, A., Valerio, F., Cimberle, M.R., Santi, L. (1982): DNA damage in liver, kidney, bone marrow, and spleens of rats and mice treated with commercial and purified aniline as determined by alkaline elution assay and sister chromatid exchange induction *Cancer Res.* 42, 2277 - 2283
- [66] Parodi, S. Zunino, A., Ottaggio, L., De Ferrari, M., Santi, L. (1983): Lack of correlation between the capability of inducing sister-chromatid exchanges in vivo and carcinogenic potency, for 16 aromatic amines and azo derivatives *Mutat. Res.* 108, 225 - 238
- [67] Pienta, R.J., Kawalek, M. (1981): Transformation of hamster embryo cells by aromatic amines *NCI Monogr.* 58, 243 – 251
- [68] Price, C.P., Typ, R.W., Marks, T.A., Paschke, L.L., Ledoux, T.A. & Reel, J.R. (1985): Teratologic and postnatal evaluation of aniline hydrochloride in the Fischer 344 rat. *Toxicol. Appl. Pharmacol.* 77, 465 - 478
- [69] Roberts, J.J., Warwick, G.P. (1966): The covalent binding of metabolites of dimethylaminoazobenzene, β -naphthylamine and aniline to nucleic acids in vivo *Int. J. Cancer* 1, 179 - 196
- [70] Rosenkranz, H.S., Poirier, L.A. (1979): Evaluation of the Mutagenicity and DNA-Modifying Activity of Carcinogens and Noncarcinogens in Microbial Systems *J. Natl. Cancer Inst.* 62, 873-892
- [71] Rosenkranz, H.S., Pet-Edwards, J., Chankong, V., Haimes, Y.Y. (1984): Letter to the editor *Environ. Mutagen.* 6, 631 – 632 (1984)
- [72] Sakagami, Y., Yamazaki, H., Ogasawara, N., Yokoyama, H., Ose, Y., Sato, T. (1998): The evaluation of genotoxic activities of disinfectants and their metabolites by umu test *Mutat. Res.* 209, 155 - 160
- [73] Sasaki, Y.F., Fujikawa, K., Ishida, K., Kawamura, N., Nishikawa, Y., Ohta, S., Satoh, M., Madarame, H., Ueno, S., Susa, N., Matsusaka, N., Tsuda, S. (1999): The alkaline single cell gel electrophoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by the IARC and U.S. NTP *Mutat. Res.* 440, 1 - 18
- [74] Sasaki, Y.F. et al. (2000): *Crit. Rev. Toxicol.* 30, 629 – 799
- [75] Shahin, M.M. (1989): Studies on the mutagenicity of aniline in association with norharman in the *Salmonella/mammalian* microsome assay *Int. J. Cosmet.* 11, 129 - 140

- [76] Shepson, P.B., Kleindienst, T.E., Edney, E.O., Namie, G.R., Pittman, J.H., Claxton, L.D., Cupitt, L.T., Perry, E. (1985): The mutagenic activity of the photooxidation products of C₆H₅NH₂/C₂H₄/NO_x mixtures J. Environ. Sci. Health, A 20, 503 - 519
- [77] Simmon, V.F. (1979): In vitro mutagenicity assays of chemical carcinogens and related compounds with Salmonella typhimurium J. Natl. Cancer Inst. 62, 893 – 899 and 901 - 909
- [78] Styles, J.A., et al. (1980): Short-term Tests Syst. Detect. Carcinogen. Progr. Symp. 1978 (226 –238) K.H. Norpoth and R.C. Garner eds., Springer Verlag Heidelberg 1980
- [79] Sugimura, T., Nagao, M. (1981): Carcinogenic, mutagenic, and comutagenic aromatic amines in human foods Natl. Cancer Inst. Monogr. 58, 27 - 33
- [80] Suzuki, Y., Nagae Y., Ishikawa, T., Watanabe, Y., Nagashima, T. Matsukubo, K. Shimizu, H. (1989): Effect of Erythropoietin on the Micronucleus Test Environ. Mol. Mutagen. 13, 314 – 318
- [81] Suzuki, Y., Shimizu, H., Ishikawa, T., Sakaba, H., Fukumoto, M., Okonogi, H. Kadokura, M. (1994): Effects of prostaglandin E₂ on the micronucleus formation in mouse bone marrow cells by various mutagens Mutat. Res. 311, 287 – 293
- [82] Swenberg, J.A., Petzold, G.L., Harbach, P.R. (1976): In vitro DNA Damage/Alkaline Elution Assay for Predicting Carcinogenic Potential Biochem. Biophys. Res. Commun. 72, 732 – 738
- [83] Swenberg, J.A. (1981): Utilization of the Alkaline Elution Assay as a Short-Term Test for Chemical Carcinogens Short-Term Tests Chem. Carcinog. 48 - 58
- [84] Takehisa, S., Kanaya, N. (1982): SCE induction in human lymphocytes by combined treatment with aniline and norharman Mutat. Res. 101, 165 - 172
- [85] Thoda, H., Tada, M., Sugawara, R., Oikawa, A. (1983): Actions of amino-β-carbolines on induction of sister-chromatid exchanges Mutat. Res. 116, 137 - 147
- [86] Topham, J.C. (1980a): The detection of carcinogen-induced sperm head abnormalities in mice Mutat. Res. 69, 149 - 155
- [87] Topham, J.C. (1980b): Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat. Res. 74, 379 - 387
- [88] Totsuka, Y., Hada, N., Matsumoto, K.-L., Kawahara, N., Murakami, Y., Yokohama, Y., Sugimura, T., Wakabayashi, K. (1998): Structural determination of a mutagenic aminophenylnorharman produced by the co-mutagen norharman and aniline Carcinogen. 19, 1995 - 2000
- [89] Vlachos, D.A. (1989): Mouse bone marrow micronucleus assay of aniline (report no. 263-89; DuPont HLR 263-89) Haskell Lab. Toxicol. Ind. Med., Newark
- [90] Wangenheim, J., Bolcsfoldi, G. (1988): Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds Mutagenesis 3, 193 - 205

- [91] Weinberger, M.W., Albert, R.H., Montgomery, S.B. (1985): Spleen toxicity associated with splenic sarcomas in rats fed high doses of D & C red. No. 9 or aniline hydrochloride *J. natl. Canc. Inst.* 75, 681 – 687
- [92] Westmoreland, C., Gatehouse, D.G. (1991): Effects of aniline hydrochloride in the mouse bone marrow micronucleus test after oral administration *Carcinogenesis* 12, 1057 - 1059
- [93] White, F.R., Eschenbrenner, A.B., White, J. (1948): Oral administration of p-aminodimethylaniline, aniline, p-aminoazobenzene and the development of tumors in rats. *Unio Int. Contra Cancrum Acta* 6, 75 – 78
- [94] Williams, G.M. (1980): DNA repair and mutagenesis in liver cultures as indicators in chemical carcinogen screening *ADV. Mod. Environ. Toxicol.* 1, 273 - 296
- [95] Wilmer, J.L. Kligerman, A.D., Erexson, G.L. (1981): Sister chromatid exchange induction and cell cycle inhibition by aniline and its metabolites in human fibroblasts *Environ. Mutagen.* 3, 627 - 638
- [96] Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., MacGregor, J.T. (2000): Micronucleated Erythrocyte Frequency in Peripheral Blood of B6C3F1 Mice from Short-Term, Prechronic, and Chronic Studies of the NTP Carcinogenesis Bioassay Program *Environ. Mol. Mutagen.* 36, 163 - 194
- [97] Yoshimi, N., Sugie, S., Iwata, H., Niwa, K., Mori, H., Hashida, C., Shimizu, H. (1988): The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes *Mutat. Res.* 206, 183 - 191

Table 1a:**Aniline: Overview on in vivo micronucleus tests with mouse and rat bone marrow cells**

Spec.	Type of expos.	Sampl. times (h)	Dose range (mg/kg)	LOED ^{a)} (mg/kg)	Max. MN freq. (neg.co)	Genetic effect	DER ^{b)}	Ref.
mouse	2 x i.p.	6 to 48	100 – 380	380	1.43% (0.27%)	positive ^{c)}	no	(1)
mouse	1 x i.p.	24	380	380	0.31% (0.003%)	positive ^{c)}	no	(2)
mouse	1 x p.o.	24 to 48	400 – 1000	1000	0.61% (0.04%)	positive	no	(2)
mouse	2 x i.p.	24 to 48	30 <- 300	300	2.58% (0.16%)	positive	no	(3)
mouse	1 x p.o.	24	125 – 250	-	-	negative		(4)
mouse	1 x p.o.	24, 48, 72	610			negative		(5)
rat	1 x p.o.	24 to 48	215 – 500	287	0.41% (0.17%)	positive ^{d)}	yes	(6)
rat	1 x p.o.	48	500	-	0.12% (0.09%)	negative ^{e)}		(7)

- a) LOED = lowest observed effective dose
b) DER = dose-effect relationship
c) same dose negative in bone marrow metaphase study (CTL, 2001b)
d) HE staining / artefact?
e) Acridine orange staining

References:

- (1) Ashby et al., 1991
- (2) Westmoreland & Gatehouse, 1991
- (3) Vlachos, 1989
- (4) Harper et al., 1984
- (5) BG-Chemie, 1985
- (6) George et al., 1990a
- (7) CTL, 2001a

Table 1b:**Aniline: Micronucleated polychromatic erythrocytes [‰; means]**

George et al. (1990)			CTL (2001b)		
Dose (mg/kg)	24 hours	48 hours	Dose (mg/kg)	24 hours	48 hours
500	2.0	4.1	500	4.9	2.0
400	2.9	3.6	400	3.0	2.1
287	3.6	2.1	300	2.7	2.5
215	1.5	1.6			
Vehicle	1.1	1.7	Vehicle	0.9	2.0

Table 2a:**Incidence of primary spleen tumors and spleen toxicity in rats (CIIT, 1982)**

	Males				Females			
	0	10	30	100	0	10	30	100
Dose (mg/kg) Aniline-HCl ¹⁾	0	10	30	100	0	10	30	100
No. of spleen examined ²⁾	123	129	128	130	129	129	130	130
Premature deaths (total No.)	16	14	15	26	20	21	14	13
<i>Tumor type</i> [*]								
Fibrosarcoma	0	0	0	3 (1*)	0	0	0	0
Stromal sarcoma	0	0	1	21 (1*)	0	0	0	0
Capsular sarcoma	0	0	0	1	0	0	0	0
Haemangiosarcoma	0	0	0	6 (4*)	0	0	0	1
Osteogenic sarcoma	0	0	0	3 (3*)	0	0	0	0
Lymphoreticular neoplasm	0	0	0	1	0	0	0	0
<i>Other findings at week 104</i> <i>(90 animals/sex/group)</i>								
Stromal hyperplasia	1	0	0	31	0	0	0	9
Chronic capsulitis	1	1	2	62	0	4	4	70
Fatty metamorphosis	0	0	0	14	0	0	0	0

1) Equivalent to aniline doses of 0, 7, 22 and 72 mg/kg bw/d

2) Including interim sacrifices at weeks 26 (10/sex/group), 52 (10/sex/group) and 78 (20/sex/group)

* In parentheses: tumors in premature deaths

Table 2b:**Incidence of spleen tumors in rats and tumors at other sites (NCI, 1978)**

Dose groups (aniline HCl)	Males			Females		
	Control	0.3%	0.6%	Control	0.3%	0.6%
Tumors in spleen and capsule						
No. of animals with tissue examined	25	50	46	23	50	50
Sarcoma NOS	0	4	2	0	0	3
Fibroma	0	7	6	0	0	0
Fibrosarcoma	0	3	7	0	0	0
Haemangiosarcoma	0	19	20	0	1	2
Lipoma	0	0	0	0	0	1
Haemangioma	0	0	0	0	0	1
Tumors in body cavity / multiple organs						
No. of animals with tissue examined	25	50	43	24	50	50
Fibrosarcoma	0	2	8	0	1	3
Leiomyosarcoma	0	0	2	1	0	0
Haemangiosarcoma	0	0	1	0	0	0
Sarcoma NOS	0	0	1	0	0	1

Stand. Mai 2002