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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

FEB 14 1994

SUBJECT: Risk Assessment for "Chloroanilines", and Other Carcinogenic Metabolites Tox. Chem No 182

TO: HED Metabolism Committee

FROM: Reto Engler
Co-Chair HED Metabolism Committee

BACKGROUND

p-Chloroaniline, and related chloroanilines are often metabolic products of pesticides applied to crops and/or fed to test animals. p-Chloroaniline in particular raises a concern with respect to its carcinogenicity; this concern has been reflected, for example, in the risk assessment of diflubenzuron (Dimlin) in the past. In this case the parent compound has been tested adequately for its carcinogenic property and was found to be negative, however a carcinogenic risk assessment was performed on the residues of the metabolite using a Q_1* for p-chloroaniline, in addition to the regular RfD type risk assessment on the parent compound. The Q_1* was calculated to be 3.9 E-2 based on preliminary information from the NTP bio-assay (memo C.J. Nelson Feb. 16, 1988; copy attached). A revised potency calculation for p-Chloroaniline is presented below.

The HED Metabolism Committee has recently evaluated several chemicals, including Dimlin, which produce a "chloroaniline" type metabolite. From these evaluations a risk assessment policy has emerged which should be applied to these chemicals as well as to other situations where a pesticide chemical is negative in the carcinogenicity assays but is metabolized to a carcinogenic compound.

RISK ASSESSMENT POLICY

The risk assessment for pesticide chemicals which produce a "chloroaniline type" (or carcinogenic) metabolite should be

performed considering the following basic principles. However, case by case considerations should also be applied, particularly with respect to the magnitude of metabolite residues, and relative risk contribution of the metabolite to the overall risk of the pesticide chemical.

1. If the metabolite is p-chloroaniline (or another carcinogenic compound) and the parent pesticide is negative for carcinogenicity a cancer risk assessment on the metabolite should be performed using the Q_1^* for the carcinogenic metabolite. The residue level of the metabolite used in the dietary exposure estimate for the purpose risk assessment should be based on actually anticipated residues, rather than on the sensitivity of an analytical method. The possible metabolic conversion of residues of the parent compound to the metabolite by man should be considered on a case by case basis, and should be "added" to the direct dietary burden resulting form residues of the metabolite in or on plants and/or meat, milk and eggs. Special studies may be required to determine the rate of metabolic conversion of the parent chemical by man.

2. If the parent compound has been tested and has been shown to be carcinogenic and its risk assessment is performed using a low dose extrapolation method, the contribution of residues of the metabolite to the overall cancer risk is probably very small. However, this depends on the relative magnitude of residues of parent and metabolite and their potency factors. These relationships should be evaluated in the overall risk assessment and the most plausible risk assessment should be developed for the purpose of regulating the pesticide chemical. Among other considerations it should be determined whether or not the tumors observed after administering the parent compound are the same type of tumors observed when feeding the metabolite.

3. If the metabolite is not PCA per se (or another carcinogenic compound), but a chemically analogous or related compound, the metabolite should be evaluated as if it were PCA (see # 1 and 2 above) unless there is sufficient evidence that the metabolite is not carcinogenic. The "evidence of non-carcinogenicity" must be determined and articulated on a case by case basis. In case there are several known carcinogenic analogues the one with the best SAR to the metabolite in question should be used for risk assessment.

REVISED POTENCY CALCULATION FOR PCA

In 1988 the Q_1^* for PCA was calculated to be 3.9 E-2 based on osteosarcomas in the spleen of male Fisher 344 rats (see attached memo from C.J. Nelson dated February 16, 1988). The following three factors would affect the calculation of a Q_1^* as of today's date.

1. The 1998 Q_1^* was calculated based on the preliminary evaluation of the study results by the NTP, e.g. it was concluded that there

were 19/50 osteosarcomas in the spleen of the top dose male rats. In the final report the NTP concluded that a number of "... uncommon sarcomas in the spleen...(fibrosarcomas, osteosarcomas, and hemangiosarcomas)...." should be combined, therefore the tumor count in the top dose male rats is 38/50 in the final report. The doubled tumor count would make the Q_1* larger.

2. The Q_1* in human equivalents calculated in 1988 used a human bodyweight of 60 kg and body surface area correction. Today we would use a human bodyweight of 70 kg for calculating the dose equivalency based on body surface area. This difference in assumed human body weight would slightly increase the Q_1*. In fact when recalculating the Q_1* using the 70 kg human body weight and the tumor numbers used by C.J. Nelson the Q_1* would be 4.0 E-2 rather than 3.9 E-2, indeed an insignificant difference.

3. There is now a trend to combine pertinent tumor data, e.g. the same tumor in male and female animals, before calculating the potency factor, whereas previously several Q_1* based on individual tumor sets may have been calculated and "averaged", for example by determining the geometric mean. In the PCA study some female rats also had these very uncommon tumors in the spleen and showed the same non-neoplastic effect, i.e. fibrosis of the spleen as did the males. The incidence of non-neoplastic lesions were the same in males and females, however, the numbers of sarcomas were much lower in females. Nevertheless, in this case the combination of the results in males and females is justified because of the very uncommon nature of the tumor in both sexes and the similar non-neoplastic effects observed in both sexes. This combination of data sets is expected to reduce the Q_1* because 1. there were less tumors in females than in males and 2. because the use of more animals (100 versus 50) increased the confidence in the Q_1* value by reducing the upper 95% confidence limit. Moreover, the fit of the data to the multistage model was significantly improved by combining the tumor data. The fit for the combined data was 0.93 (nearly perfect), whereas the fit for the male rat splenic tumor data alone was only 0.5.

The summary of the NTP report (TR 351; July 1988) is attached. Overall this study seems of very good quality. Three doses were tested and the dose selection was carried out very well, i.e. there did not seem to be any excessive toxicity at the top dose which might have interfered with the interpretation of the study results. It also should be noted that PCA was positive in all the genotoxicity tests reported by NTP. There was also a tumor response in male mice (hepatocellular tumors). A Q_1* based on this tumor set was calculated in 1988 but even though it was larger then the Q_1* based on splenic tumors in the rat, it was not used for risk assessment. The reasons for this were obvious then as they are now: hepatocellular tumors in male B6C3F$_1$ mice are biologically much less

3

convincing than sarcomas (many of them metastasized) in the rat spleen. For this reason the Q_1* based on male liver tumors were not re-calculated; the original value, albeit based on a 60 kg human, is listed in the 1988 Nelson memo.

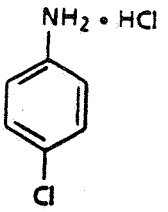
p-Chloroaniline has not been evaluated and classified by the HED peer review Committee. p-Chloroaniline also has not been verified by CRAVE and is not listed on IRIS. However, the cancer bioassay data generated by NCI and the clearly genotoxic properties of p-Chloroaniline leave very little doubt about its carcinogenic properties and warrant the calaulation of a Q_1*.

The following tumor data were subjected to the analysis by the Crump multistage model (Crump Tox Risk version 3.0).

	Splenic Tumors (Rats)			
dose (mg/kg/day)	0	2	6	18
males	0/49	1/50	3/50	38/50
females	0/50	0/50	1/50	1/50
Total	0/99	1/100	4/100	39/100

The Q_1* based on the total tumor rate was 5.9 E-2 $(mg/kg/day)^{-1}$, a somewhat higher Q_1* of 9.2 E-2 was obtained when using the tumor data in male rats only. However, for the reasons discussed above, it seems appropriate to use the Q_1* which considers the data in male as well as female rats.

cc:
BJaeger
PHutton
HPettigrew
WBurnam
LRossi
PFenner-Crisp
JKariya
MVanGemert
KDearfield
Caswell File 182



p-CHLOROANILINE HYDROCHLORIDE

CAS No. 20265-96-7

$C_6H_6NCl \cdot HCl$ Molecular weight 164.1

Synonyms: 1-amino-4-chlorobenzene hydrochloride; 4-chlorophenylamine hydrochloride; 4-chlorobenzenamine hydrochloride

ABSTRACT

p-Chloroaniline has a large production volume and is used as a dye intermediate. Toxicology and carcinogenesis studies of *p*-chloroaniline (greater than 99% pure) were conducted by administering *p*-chloroaniline hydrochloride in water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Vehicle controls were given deionized water by gavage. All doses were calculated as *p*-chloroaniline; the chemical was administered as the hydrochloride after dissolution in water containing molar equivalents of hydrochloric acid. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells. Hematologic parameters were measured at the end of the 13-week studies and at 6, 12, 18, and 24 months in the 2-year studies. Supplemental studies of the distribution and disposition of *p*-chloroaniline were conducted in male F344 rats.

Sixteen-Day and Thirteen-Week Studies: In the 16-day studies, male and female rats and mice received 25, 50, 100, 200, or 400 mg/kg of body weight. The vehicle controls received deionized water. All rats and mice that received 200 or 400 mg/kg died during the first 6 days of the studies. Some deaths occurred in each of the lower dose groups of mice. Splenic enlargement was observed at necropsy in rats administered 25, 50, or 100 mg/kg. Congestion of the spleen and hemosiderin deposition in the renal cortical tubular epithelial cells were observed at 100 mg/kg in male and female rats. Compound-related lesions in mice included hemosiderosis of the liver Kupffer cells and congestion of the spleen.

In the 13-week studies, 10 rats of each sex were administered doses of 0, 5, 10, 20, 40, or 80 mg/kg. All male rats lived to the end of the 13-week studies. One of 10 female rats that received 80 mg/kg died from unknown causes. The final mean body weights of rats that received 80 mg/kg were 16% lower than that of vehicle controls for males and 4% lower for females. In the 13-week studies in mice, 10 animals of each sex were administered doses of 0, 7.5, 15, 30, 60, or 120 mg/kg. Deaths in mice were not related to *p*-chloroaniline hydrochloride administration. The final mean body weights of dosed and vehicle control mice were similar. In both rats and mice, no chemically related effects on organ weights were observed at necropsy, except for the spleen, which was enlarged as a function of increasing dose. Methemoglobin was increased in dosed groups and resulted in a secondary anemia, the severity of which was dose related. Compound-related lesions observed histologically, including pigmentation (hemosiderin) in the kidney, spleen, and liver and hematopoiesis in the liver and spleen, reflected the response to the hemolytic anemia and methemoglobinemia induced by *p*-chloroaniline hydrochloride.

Based on these results, groups of 50 rats of each sex were administered 2, 6, or 18 mg/kg *p*-chloroaniline hydrochloride in water by gavage, 5 days per week for 103 weeks. Groups of 50 mice of each sex were administered 3, 10, or 30 mg/kg on the same schedule.

Metabolism and Disposition Studies in Rats: The metabolism and disposition studies in F344/N rats showed that metabolic and excretory pathways were not saturated by *p*-chloroaniline administered orally at doses ranging from 0.3 to 30 mg/kg. *p*-Chloroaniline was rapidly metabolized and excreted primarily in urine with a half-life of approximately 2 hours.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally within 5% of those of vehicle controls throughout the studies. The survival of the low and mid dose groups of male rats and of the low and high dose groups of female rats was significantly greater than that of the vehicle controls (male: vehicle control, 18/49; low dose, 32/50; mid dose, 32/50; high dose, 21/50; female: 27/50; 39/50; 36/50; 37/50). The increased survival was attributed to the decreased incidences of mononuclear cell leukemia. Mean body weights of high dose male and female mice were generally within 5% of those of vehicle controls throughout the studies. The survival of the mid dose group of male mice was lower than that of the vehicle controls after week 99 (male: 43/50; 36/50; 29/50; 35/50; female: 39/50; 42/50; 44/50; 41/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Fibrosis of the spleen was increased in dosed male and high dose female rats (male: vehicle control, 3/49; low dose, 11/50; mid dose, 12/50; high dose, 41/50; female: 1/50; 2/50; 3/50; 42/50). Cellular infiltration of lipocytes (fatty metaplasia) was observed in the spleen at increased incidences in high dose rats (male: 0/49; 0/50; 0/50; 24/50; female: 0/50; 0/50; 0/50; 11/50). The incidence of uncommon sarcomas of the spleen in high dose male rats was significantly greater than that in the vehicle controls (fibrosarcomas, osteosarcomas, or hemangiosarcomas, combined: 0/49; 1/50; 3/50; 38/50). Many of these tumors metastasized to one or more sites. In female rats, one fibrosarcoma of the spleen was found in a mid dose animal, and one osteosarcoma of the spleen was found in a high dose animal. The historical incidence of splenic connective tissue sarcomas (all types) in water gavage vehicle controls is 1/298 (0.3%) for male rats and 0/297 for female rats. The historical incidence of hemangiosarcomas in water gavage controls is 0/300 for male rats and 1/297 (0.3%) for female rats.

Adrenal medullary hyperplasia was observed at an increased incidence in high dose female rats (4/50; 4/50; 7/50; 24/50). Marginally increased incidences of pheochromocytomas were seen in high dose male (13/49; 14/48; 15/48; 26/49) and female (2/50; 3/50; 1/50; 6/50) rats. The historical incidence of pheochromocytomas in water gavage vehicle control male F344/N rats is 121/299 (40% ± 16%); the historical incidence in water gavage vehicle control female F344/N rats is 20/295 (7% ± 2%).

The incidences of mononuclear cell leukemia in dosed male and female rats were lower than those in vehicle controls (male: 21/49; 3/50; 2/50; 3/50; female: 10/50; 2/50; 1/50; 1/50). The incidences of malignant lymphomas in dosed male and female mice were lower than those in vehicle controls (male: 10/50; 3/49; 9/50; 3/50; female: 19/50; 12/50; 5/50; 10/50).

Hematologic and methemoglobin measurements were made on blood samples collected from 15 randomly selected male and female rats per dose group at 6, 12, 18, and 24 months. In general, the high dose group at various intervals showed mild hemolytic anemia and dose-related increases in methemoglobin.

In rats, compound-related nonneoplastic lesions were seen histopathologically in the bone marrow, spleen, and liver. These lesions included bone marrow hyperplasia, hepatic hemosiderosis, and splenic fibrosis and suggest compound-related effects on the hematopoietic system in general, the erythropoietic system specifically, and mesenchymal cells in the spleen.

In male mice, the incidence of hemangiosarcomas of the liver or spleen in high dose male mice was greater than that in the vehicle controls (4/50; 4/49; 1/50; 10/50). The historical incidence of hemangiomas or hemangiosarcomas at all sites (combined) in water gavage vehicle control male B6C3F$_1$ mice is 11/350 (3% ± 3%).

The incidences of hepatocellular adenomas or carcinomas (combined) were increased in dosed male mice (11/50; 21/49; 20/50; 21/50), primarily due to increased incidences of hepatocellular carcinomas (3/50; 7/49; 11/50; 17/50). Hepatocellular carcinomas metastasized to the lung in 1/50 vehicle control, 1/49 low dose, 2/50 mid dose, and 9/50 high dose male mice. The historical incidence of hepatocellular neoplasms in water gavage vehicle controls is 106/347 (31% ± 6%).

Genetic Toxicology: *p*-Chloroaniline was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of exogenous metabolic activation; no increase in revertant colonies was observed in strains TA97, TA1535, or TA1537. *p*-Chloroaniline induced trifluorothymidine (Tft) resistance in mouse L5178Y lymphoma cells with and without metabolic activation. In cultured CHO cells, treatment with *p*-chloroaniline produced significant increases in sister chromatid exchanges (SCEs) both with and without metabolic activation (S9); chromosomal aberrations were significantly increased only in the presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of *p*-chloroaniline have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year water gavage studies, there was *clear evidence of carcinogenic activity** of *p*-chloroaniline hydrochloride for male F344/N rats, as indicated by increased incidences of uncommon sarcomas of the spleen. Pheochromocytomas of the adrenal gland may also have been associated with chemical administration. There was *equivocal evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for female F344/N rats, as indicated by the presence of uncommon sarcomas of the spleen in one mid and one high dose animal and the increased incidence of pheochromocytomas of the adrenal gland. There was *some evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for male B6C3F$_1$ mice, as indicated by increased incidences of hepatocellular neoplasms and of hemangiosarcomas of the liver or spleen. There was *no evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for female B6C3F$_1$ mice administered 3, 10, or 30 mg/kg by gavage for 2 years.

The incidences of mononuclear cell leukemia in male and female rats and of malignant lymphomas in male and female mice were decreased by administration of *p*-chloroaniline hydrochloride. Compound-related splenic fibrosis was present in male and female rats.

---

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 7.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 10.

SUMMARY OF THE TWO-YEAR GAVAGE STUDIES OF *p*-CHLOROANILINE HYDROCHLORIDE AND GENETIC TOXICOLOGY STUDIES OF *p*-CHLOROANILINE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
2, 6, or 18 mg/kg *p*-chloroaniline in acidified water, 5 d/wk; vehicle controls received deionized water	2, 6, or 18 mg/kg *p*-chloroaniline in acidified water, 5 d/wk; vehicle controls received deionized water	3, 10, or 30 mg/kg *p*-chloroaniline in acidified water, 5 d/wk; vehicle controls received deionized water	3, 10, or 30 mg/kg *p*-chloroaniline in acidified water, 5 d/wk; vehicle controls received deionized water
Body weights in the 2-year study			
Dosed groups within 5% of vehicle controls	Dosed groups within 5% of vehicle controls	Dosed groups within 5% of vehicle controls	Dosed groups within 5% of vehicle controls
Survival rates in the 2-year study			
18/49; 32/50; 32/50; 21/50	27/50; 39/50; 36/50; 37/50	43/50; 36/50; 29/50; 35/50	39/50; 42/50; 44/50; 41/50
Nonneoplastic effects			
Fibrosis of the spleen (3/49; 11/50; 12/50; 41/50)	Fibrosis of the spleen (1/50; 2/50; 3/50; 42/50)		
Neoplastic effects			
Sarcomas of the spleen (0/49; 1/50; 3/50; 38/50); pheochromocytomas of the adrenal gland (13/49; 14/48; 15/48; 26/49)	Sarcomas of the spleen (0/50; 0/50; 1/50; 1/50); pheochromocytomas of the adrenal gland (2/50; 3/50; 1/50; 6/50)	Hepatocellular adenomas or carcinomas (combined) (11/50; 21/49; 20/50; 21/50); hemangiosarcomas (4/50; 4/49; 1/50; 10/50)	None
Level of evidence of carcinogenic activity			
Clear evidence	Equivocal evidence	Some evidence	No evidence
Other considerations			
Decreased incidences of leukemia (21/49; 3/50; 2/50; 3/50)	Decreased incidences of leukemia (10/50; 2/50; 1/50; 1/50)	Decreased incidences of lymphomas (10/50; 3/49; 9/50; 3/50)	Decreased incidences of lymphomas (19/50; 12/50; 5/50; 10/50)
Genetic toxicology			
Salmonella (gene mutation): Positive with S9; negative without S9; Mouse L5178Y/TK$^{+/-}$ (Tft resistance): Positive with and without S9		CHO Cells in Vitro: SCE: Positive with and without S9; Aberration: Positive with S9; negative without S9	

8