KOR. J. ENVIRON. TOXICOL.
Vol. 15, No. 1~2, 31~37 (2000)

Initial Risk Assessment of Acetanilide with Respect to Human Health

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아세트아닐리드의 초기 인체위해성 평가

이서래, 최선주, 이미경, 남우경, 정선화,[1] 석금수,[1] 박광식,[2] 김 균, 김용화

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요 약

아세트아닐리드는 그의 생산 및 이용 공장에서 환경으로 방출된 다음 인체에 노출될 수 있다. 아세트아닐리드는 진통효과를 나타내는 것으로 알려져 과도 노출시에는 건강에 부작용을 초래할 수 있다.

EUSES 시스템에 의하면 아세트아닐리드는 지역노출의 경우 6×10^{4}을 초과하는 높은 MOS 값(안전성 마진)을 보여주어 공중보건상 충분히 안전한 것으로 나타났다. 국지수준(작업장)에서 경피노출에 의한 MOS 최저 값은 3×10^{-4}으로 추정되었지만 작업장에서 개인장비나 환기와 같은 예방조치에 의하여 그 위험을 부분적으로 경감시킬 수 있다. 아세트아닐리드는 분진 흡입하는 작업자에게 위험 가능성이 나타날 수 있다.

작업장에서 건강보호를 위해서는 산업보건 측면에서의 안전함이 증명될 수 있도록 반복투여독성, 생식독성 및 발육독성에 관한 자료가 보완되어야 할 것이며, 따라서 이에 대한 실험이 진행되어야 할 것으로 사료된다.

INTRODUCTION

The investigation of the safety of so many chemicals currently in commerce is a daunting global challenge that can only be met if approached in a systematic way. This enormous task has been undertaken by the OECD programme to investigate high production volume (HPV) chemicals in a co-operative way.[1)]

Among chemicals selected for investigation by Korean side from the year of 1999, acetanilide is the first to collect pertinent information, review the data quality and attempt an initial risk assessment. This study was intended to make an initial health risk assessment of acetanilide following the OECD manual with limited data available. This case study is expected to trigger the attempt to identify, prioritize, and manage chemicals of human health in the go-

vernment, the industry, and the public. The word "initial" was used because the data used for the risk assessment are not complete set of data generated solely by experiment.

MATERIALS AND METHODS

1. Data search

All the data sources used in this study were given in the previous paper.[2)]

2. Prediction of data

It was found that many data needed for the risk assessment were not available. Therefore, several programs were used for the prediction of the parameters. These were as follows:

- EUSES (European Union System for the Evaluation of Substances, Ver. 1.00)
- EPIWIN (EPIWIN v2.2, SRC-EPI for Microsoft Windows, 1994~1996)

RESULTS

1. Exposure

1) National production and use categories

Total production of acetanilide in Korea was about 2,300 metric tons/year and the import into Korea was less than 1% of the produced in 1998. Of the 2,300 metric tons consumed in Korea, most is used as an intermediate for the syntheses of pharmaceuticals and dyes. Less than 0.5 ton is used as a stabilizer in hydrogen peroxide solution. Most of the acetanilide is consumed by one company in Korea, which is the only producer of the chemical.

2) Occupational exposure

Although limited monitoring data indicate that non-occupational exposure can occur from the ingestion of contaminated drinking water, the most probable human exposure would be occupational exposure, through dermal contact or inhalation at workplaces where acetanilide is produced or used. Until now, there are no OSHA, ACGIH, NIOSH standards or regulations available on occupational exposure level of acetanilide. However, it is well known to produce cyanosis in some humans when taken repeatedly, possibly due to formation of sulfhemoglobin. NIOSH (National Occupational Exposure Survey 1981~1983) has statistically estimated that 9,000 workers (6,100 of these are female) are potentially exposed to acetanilide in USA.

3) Consumer exposure

Most of acetanilide is used for the synthesis of pharmaceuticals and dyes, and only 0.05% is consumed as a stabilizer of hydrogen peroxide solution. It can be postulated that there would be virtually no direct consumer exposure by acetanilide.

4) Indirect exposure via the environment

The amount of release from manufacturing site of acetanilide to environmental compartments is relatively small.[2)] According to the Fugacity model level I (EQC model), 98.6% of released acetanilide is distributed in water. PEC for surface water was estimated to be 2.5×10^{-2} mg/l (local) or 9.1×10^{-5} mg/l (regional), as the worst-case. The concentration of acetanilide in drinking water processed from surface water should be lower than the PEC. The daily intake through such drinking water is calculated as $< 8.3 \times 10^{-4}$ mg/kg bw/day (based on 2 L/day, 60 kg body weight and worst local PEC).

Using the bioconcentration factor of 4.5 in aquatic organisms, the concentration of acetanilide in fish can be calculated as follows:

$$PEC_{fish} = 2.5 \times 10^{-2}\,(mg/l) \times 4.5 = 1.13 \times 10^{-1}\,mg/kg$$

As the daily intake of fish in Korea is estimated to be 90 g for 60 kg bw person, a daily intake of acetanilide will be 1.6×10^{-4} mg/kg bw/day. The combined intake of acetanilide through drinking water and fish becomes approximately 1×10^{-3} mg/kg bw/day.

2. TOXICITY

1) Toxicokinetics, metabolism and mechanism of action

Acetanilide is, in fact, converted to its phenolic metabolite in the body which gives it an analgesic effect, but some is converted to aniline (aminobenzene) which is highly toxic. It is very well known that numerous drugs and chemicals derived from aniline are known to cause various toxic effects such as methemoglobinemia, hemolysis and hepatotoxicities. Studies have shown that acetanilide induces the potential hepatic damages in various animal species such as disruption of hepatic enzyme functions. Certainly, the exposure of acetanilide to human body could lead to not only liver damage but also to possible damage in major organs in the human body.

For toxicokinetics of acetanilide, it was found that single dose of 10 mg/kg of acetanilide resulted in 191.5±27.8 min. of blood plasma half-life of acetanilide in 25 subjects and 14.1±2.8 liter/h of metabolic clearance rate.[3]

2) Acute toxicity

The acute effects of acetanilide exposure have been examined in mice, rats, guinea pigs, rabbits, cats, and dogs. The adverse effects observed in laboratory animals were ptosis, lethargy, abnormal gait, lacrimation, sedation, narcosis and even paralysis and death after administration.[4,5] The LD_{50} in animal studies was in the range of 886~2,033 mg/kg by oral and 500~715 mg/kg by i.p., differing by species and investigators. Regardless of the species examined, these adverse effects are generally similar to the signs of central nervous system depression seen in the case of human intoxication.[6] The study after fatal dose administration of acetanilide (1,000 mg/kg) in rats revealed the formation of methemoglobin and sulfhemoglobin in blood sample. Even the death of animals shortly after the blood sample was taken was reported.[7] It was reported that the factory workers exposed to acetanilide in India had been suffering from cyanosis after duty hours. Although there was no apparent signs or symptoms, workers complained of chest pains, giddiness, epigastric pain and showed strongly colored urine.[8] These symptoms are very similar to the consequences of mild poisoning of acetanilide. One of the aspects of acute poisoning seems to be related with neurological disturbance in the CNS and can result in suicidal tendencies.[6] It was also reported that contact, inhalation or ingestion might cause an eczematous eruption of the skin and dermatitis.[9]

The LDLo (p.o.) value of acetanilide in human exposure was known to be 59 mg/kg. The oral lethal dose (human) was reported at the level of 5~50 mg/kg (grade 5) or 50~500 mg/kg (grade 4).[10]

3) Repeated dose toxicity

The *in vitro* study of the hematological and blood biochemistry in monkeys revealed the formation of methemoglobin and sulfhemoglobin in 2 Mangabey monkeys with 540 mg/kg/day of acetanilide for several days.[7] Another study using Sprague-Dawley rats showed anemia, splenomegaly and dark discoloration of the livers.[11] Moreover, from the study using cats, LD_{100} was reported at 125 mg/kg with extensive hemorrhage in the kidneys and mottled livers.[12] Most animal studies reveal the chronic toxicity of acetanilide as methemoglobin and sulfhemoglobin formation, and anemia. Although acute toxicity seems to be very low, methemoglobin and some hyperplasia of the bone marrow are the common signs of chronic intoxications of acetanilide when it is administered at high doses.[13]

Most of the animal studies are very well correlated with human toxicity data in chronic exposure to acetanilide. One study reported that the continued use of acetanilide as a drug leads to chronic poisoning characterized by gastroenteric disturbances, cardiac dysfunction, drowsiness, hemolytic anemia, methemoglobinemia, reticulocytosis, cyanosis, antipyresis, acute renal failure and collapse as well as advanced degenerative changes in the kidney.[13)-15] Even mild poisoning with acetanilide was known to cause headache, weakness, hypotension, nausea and vomiting, but more severe poisoning would lead to depression of cardiac and smooth muscle in human.[6]

Several repeated dose studies were carried out with rodents, cat, dog and monkey. However, it was not

easy to estimate NOAEL because the test conditions had not been planned to generate appropriate subchronic or chronic toxicity data. Furthermore, no recent data on repeated toxicity are available.

4) Reproduction/developmental toxicity

Only one study using mice showed the depressant effect on reproduction and litter raising up to 4th generation at 125 mg/kg. Since there was a significant reduction of survival in the 3rd and 4th generation, and insufficient number of offsprings was born to continue the 5th generation, the study had to be stopped.[16] In addition to the depressant effect on reproduction and litter raising, reduction of survival in the 2nd generation, retardation of growth, deterioration of condition, methemoglobin formation and cyanosis were the other signs shown by the surviving animals.

However, the study was originally carried out for chronic toxicity and not intended for developmental toxicity. Therefore, the specific methodological details of the study do not meet the requirements of OECD test guidelines such as using inbred mice, limited number of dose, number of mating successfully, number of live births, etc. Under these circumstances, the result could not be used as a qualified data for hazard assessment, such as generating NOAEL value.

For the teratogenicity study of acetanilide, an *in vitro* test was performed using *Drosophila* embryonic cell cultures. It was found that acetanilide showed no teratogenic effect in the assay.[17] However, it is inappropriate to use *Drosophila* to determine the potential for developmental toxicity. Accordingly, a further study using rodents is needed.

5) Genetic toxicity

Several studies showed that acetanilide was found to be non-mutagenic to *Salmonella typhimurium* with or without metabolic activation.[18)-23)] Most of the studies using chromosomal aberration, recombination assay and point mutation showed negative results. However, one study revealed acetanilide as a mutagen in *Bacillus subtilis* recombination assay.[20]

Several in vivo studies showed positive or negative results. Among 4 cytogenetic tests, 2 studies with rats showed positive results.[20),24)] In a micronucleus test, the result was positive following methods similar to the current OECD guidelines.[25] Therefore, it could be summarized that acetanilide might be a potential genotoxic material based on the tests using in vitro and in vivo methods.

6) Irritation/sensitization

Although there are a few data related to skin or eye irritation, one study showed slightly irritating characteristics of acetanilide in the eyes of rabbit under the GLP condition.[26] Related to this animal study, human exposure to acetanilide resulted in eczematous eruption of the skin by contact, inhalation or ingestion[9] with loss of vision and semi-dilated unreactive pupils following ingestion of acetanilide in humans.[15]

7) Carcinogenicity

Information on carcinogenicity is available from the studies performed in rats, mice and hamsters. Male and female of several animal species were subjected to the study. However, carcinogenicity studies showed no evidence of tumor like liver tumor, bladder tumor, mammary tumor, etc.[27),28)] Even the 4th generation studies using mouse strains of ABC-A revealed that there was no appearance of tumor in the mammary gland.[16] However, these studies would not lead to a final conclusion on non-carcinogenicity of acetanilide.

8) Summary of toxicity data

Acetanilide is known to produce an analgesic effect after metabolic conversion to phenolic compounds in a biological system. Its action as a painkiller is related to the inhibition of the synthesis of prostaglandin, which is a vasal dilator causing headaches and hypotension, and this process is involved with hormones and nervous system. The results of the animal and human exposure studies ascertain the acetanilide toxicity to human and it is not surprising that the old painkiller, acetanilide, was phased out of

the pharmaceutical market.

Based on the results of animal studies such as acute oral, chronic, genetic and other studies, acetanilide should be considered as moderately toxic chemical from the human health aspect. The main adverse effect of acetanilide in acute toxicity was the depression of the central nervous system. Other adverse effects were found in the blood system such as cyanosis due to the formation of methemoglobin and sulfhemoglobin. Acetanilide was also found to be an irritant to the eyes. Toxic effects of acetanilide toward animals and human are summarized in Table 1.

In summary, acetanilide poses adverse effects on human health. Inhalation of acetanilide dust or vapors may cause irritation to the respiratory tract, mucous membranes and skin. When it is ingested, highly toxic effects in human can occur. Even several grams may produce poisoning with circulatory collapse, cold extremities, paleness and feeble rapid pulse. Above all, cyanosis is the prominent effect. With skin and eye contact, it causes irritation to skin. Symptoms include redness, itching, and pain. Lengthy chronic exposures may affect the ability of blood to carry oxygen (methemoglobinemia), resulting in bluish discoloration of the lips and tongue (cyanosis).

3. INITIAL RISK ASSESSMENT FOR HUMAN HEALTH

For the purpose of risk assessment, NOAEL value based on repeated dose toxicity at least should be available. Since appropriate NOAEL value for acetanilide is not avalilable at the moment, quantitative risk assessment could not be attempted. However, a preliminary trial was made with limited data to foresee needed area of further studies.

For human health, both inhalation of vapor/particle and skin exposure are the main routes of exposure in work place, and oral exposure of acetanilide is not considered to be significant under normal working conditions. In Table 2 and 3, the corresponding MOS (margin of safety) values for the dermal and inhalation exposure are given for the work places and for the general public in the local/regional environments, respectively.

For the occupational exposure, acute oral LD_{50} value for mouse, 1,210 mg/kg, was adopted in running the EUSES model. For the local and regional exposures toward the general public, subchronic oral

Table 1. Summary of toxic effects of acetanilide relevant to work places

Acute Toxicity	LD_{50} (rat, oral) = 2,033 mg/kg, LD_{50} (mouse, oral) = 1,210 mg/kg,	
Corrosiveness/Irritation	Eye : Slightly irritating, Dermal: Irritating	
Skin Sensitization	No case report	
Repeated Dose Toxicity	Methemoglobin, Cyanosis, Anemia	
Mutagenicity	Genetoxicity was unlikely to be expressed in Ames test. Positive in micronucleus and cytogenetic tests with rodents	
Carcinogenicity	No carcinogenicity was found	
Developmental Toxicity & Teratogenicity	No developmental toxicity and teratogenicity were found in *Drosophila*	
Specific Toxicity	Neurotoxicity	Neurobehavioral dysfunction CNS depressant
Human Exposure	Work place	LDLo = 59 mg/kg
	Acute poisoning	Cyanosis, anemia, vertigo, visual disturbance, circularly collapse, coma and death
	Mild poisoning	Headache, weakness, hypotension
	Human dermal exposure	Dermatitis
	Inhalation or ingestion	May cause an eczematous eruption of skin

Table 2. MOS of acetanilide in the work places, as estimated by EUSES system

Protection	Type of processing	Inhalation		Dust
		Dermal	Vapour	
Without protective equipment	Filling	2.96	0.033~0.333	0.0003~0.0013
	Dry crushing and grinding	2.96	0.008~0.033	0.030~0.298
With protective** equipment	Filling	2.96	0.333~0.833	-*
	Dry crushing and grinding	2.96	0.167~0.833	-*

* MOS becomes the infinite. Since potential dermal uptake = 0,
** Protective equipment includes ventilation and preventive measures to avoid direct dermal contact.

Table 3. MOS of acetanilide for the general public in local and regional environments, by EUSES system

Exposure media	Local exposure		Regional exposure
	Formulation	Processing	
Exposure via air	382	3.19×10^5	1.38×10^8
Total exposure via all media	1.67	6.43×10^2	6.56×10^4

LOAEL value of 0.5 mg/kg, which was obtained by applying an uncertainty factor of 100 toward the acute oral LDLo 59 mg/kg for humans, was adopted. According to the OECD context, uncertainty factor was not considered so that MOS higher than 1 is regarded as safe for this initial assessment.[1)]

For the general public, there were no local and regional risks, giving MOS values higher than 1 in any case as estimated using the EUSES system. In the work places, MOS value for the dermal exposure without protective equipment was about 3×10^{-4}, but the value becomes infinite with protective equipment on the assumption that potential dermal uptake is zero. In the case of inhalation exposure, the value of MOS was also found to be very low and it is still below 1 with protective equipment. A special attention should be paid to mitigate the risk. Actually, the precautions in the work places have already been recommended and are in action.

Based on the lower value of MOS in work places, it can be concluded that acetanilide may pose some potential risk toward occupational hazard by inhalation even if acetanilide is handled with protective equipment including ventilation and protection to avoid direct dermal contact, according to the OECD context. In order to raise the MOS value above 1, it is recommended to accumulate either exposure data in the work places or acute inhalation toxicity data in the future, to replace the default uncertainty factor used in the EUSES model.

ABSTRACT

Acetanilide may be released into the environment through air and wastewater from its production and use sites and exposed to human. Acetanilide is known to produce an analgesic effect and may pose adverse effects on human health by overly exposure.

According to the EUSES system, acetanilide showed a high MOS (Margin of safety) value exceeding 6×10^4 on a regional exposure, which is safe enough for public health. Whereas the lowest MOS value in dermal exposure was estimated as 3×10^{-4} on a local basis (workplace), the risk could be partly counteracted by taking preventive measures such as using mask and globes and good ventilation in the work places. Acetanilide may pose a potential risk for workers by dust inhalation.

For the sake of health protection in the work places, additional data should be accumulated with respect to repeated dose toxicity, reproduction toxicity and developmental toxicity, etc. It is, therefore, recommended that acetanilide should be a candidate for further work to supplement the lacking data until it is proved to be safe in the occupational health aspects.

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