Asian J. Exp. Sci., Vol. 22, No. 3, 2008; 317-324

Effects of Oral Exposure of Acrylamide on Plasma Levels of Thyroid Hormones and Haematological Parameters in the Swiss Albino Mice

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Abstract : Effects of acrylamide on thyroid hormone levels and haematological parameters in Swiss albino mice were investigated by exposing them with 3 dose levels set below LD_{50}. Mortality in animals was nil with a significant decrease in the mean body weight gain. The various toxic morphological effects noted were sluggish appearance, bizarre behaviour, stunted growth, reduced limb movements, diminished food and water consumption. The dose dependent decrease in the T_3 and T_4 levels and a consequent increase in TSH was observed in the order of $E_1 < E_2 < E_3$. The thyroid hormones control the skeletal and mental growth along with cell respiration, thus the above morphological toxic effects can be synchronized with the above results of T_3, T_4 and TSH. A decreasing pattern was also found in the haematological parameters like Hb content, erythrocyte count and haematocrit value. Since acrylamide is electrophilic in nature, it binds with cystein residues and forms adducts with Hb. Therefore, it might have reduced the Hb content and also the erythrocyte counts in the experimental animals. In this way, acrylamide completely distrubed the equilibrium of haematological and thyroid hormonal status. Since this chemical has swiftly invaded in our daily lives, a further corollary study is required to furnish the science world with better understanding regarding the usage of acrylamide.

Key words : Swiss albino mice, Thyroid gland, Thyroid Stimulating Hormone, Thyroxine, Haemoglobin.

Introduction

Acrylamide (CH-CO-NH2), commercially available as a crystalline solid or a 30% -50% solution in water is a widely known industrial chemical. There are various synonyms of acrylamide as —2 propenamide, acrylamide monomer, acrylic acid amide, acrylic amide, ethylene carboxamide etc. The two major forms of acrylamide are monomer and polymer, monomer form is highly toxic while the polymer form is reported to have very low toxicity or no toxicity (Bikales, 1973; Geise, 2002; Konings *et. al.*, 2003; Richmond and Borrow, 2003; Tyl and Crump, 2003;Vattem and Shetty, 2003).

The major uses of acrylamide monomer are in the production of high molecular mass polymers or of cationic and anionic copolymers which are widely used :

- In effluent and sludge treatment as flocculants and coagulants.
- In crude oil recovery processes as viscosity modifiers.
- In paper industry as binders, dewatering aids and paper strengthening agents.
- In drilling mud as retention and draining aids.
- In paints, coatings, toiletries and cosmetics as thickeners and binders.
- In foundry sand as moisture retention additives .

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These polymers and copolymers also play various roles in textile processing, photography, dyeing, electrophoresis, animal feed, pharmaceuticals , adhesives , tapes and gels (Giese, 2002; Simonne and Archer, 2002; Croll *et. al.*, 1974; Conway *et. al.*, 1979; Brown *et. al.*, 1980; Bikales, 1973; Bluementhal *et. al.*, 1995; Dearfield *et. al.*, 1988; EC, 2000; American Cyanamid, 1961, ACGIH 1986; US EPA 1998). The largest use of polyacrylamide is in treating municipal drinking water and industrial waste water to remove suspended solids before discharge, reuse or disposal (Croll *et al*., 1974).

Acrylamide is also a component of tobacco smoke, which indicates that it can be formed by heating of biological materials like cigerette etc. (EC, 2000; Marsh *et. al* .,1999; IARC 2002; PHS 2004). The monomer form is a well known neurotoxicant and has shown to affect central and peripheral nervous systems in both acute and chronic exposures (Kuperman *et al*., 1958). The nuerotoxic effects reported are drowsiness, hallucinations, ataxia, impaired hind limb movements, convulsions, diffused damage to different sections of the nervous system, lysis in the cerebellum neurons and tibial nerve degeneration (Novikov *et. al.*, 1979; HSDB 1994; Crofton *et. al.*, 1996; Kuperman 1958; Mc Collister *et. al.*, 1996; Sobel *et. al.*,, 1986; Tilson, 1981; Bachmann *et.al.*, 1992; Myers and Maccum, 1991; CERHR, 2004; Gold *et. al.*, 2004).

The non neurotoxic effects of this dreadful substance includes genotoxicity, reproductive toxicity and carcinogenicity. Genotoxicity comprises of chromosomal aberrations, dominant lethality, sister chromatid exchanges and unscheduled DNA synthesis (Deorge *et. al.*, 2005 a,b; Ghanayem *et. al.*, 2005; Glatt *et. al.*, 2005; Husoy *et. al.*, 2005; Paulsson *et. al.*, 2002, 2003; CERHR 2004 a,b; Allen *et. al.*, 2004). Reproductive toxicity includes testicular atrophy, decreased fertility, reduced numbers of spermatozoa, degenerating spermatids and spermatocytes and multinucleate gaint cells (Smith *et. al.*, 1996; Miller *et. al.*, 1982) and carcinogenicity has been reported to be forming tumors in organs like thyroid gland, scrotum, tissues of the central nervous system, mouth, uterus, clitoris and mammary glands in males and females of mice and rats (Johnson *et. al.*, 1986; Rice Jerry, 2005; Friedman *et. al.*, 1995).

US EPA and IARC have classified acrylamide as B_2, a probable carcinogen and as 2B, a possible human carcinogen respectively (Dearfield *et. al.*, 1988; Friedman *et. al.*, 1995). The other additional toxicological effects reported are depletion of adipose tissues, decreased liver and kidney, mottled lungs, atrophy of skeletal muscle, distension of urinary bladder, thickening of stomach and decrease in RBC count and packed cell volume (Miller *et. al.*, 1982). Acrylamide also crosses placenta and passes in a significant concentration to the developing foetus leading to direct pre-natal and post natal changes in rodent off springs (Dearfield *et. al.*, 1988; Edwards, 1976); it ascertains the fact that not only the live forms directly exposed to this chemical are affected, but the still to be borns too can not remain aloof from the same, making it an important researchable substance.

Thyroid gland holds a key importance among the endocrine glands as it works for the normal body metabolism, growth and development including aging and the regulation of basal metabolic rate (BMR) (Capen *et. al.*, 1991; Lu and Anderson, 1994), the activity of this gland is commonly determined by its hormone secretion rate (Lu and Anderson, 1994). Since the uses and biological effects of acrylamide are varied along with its manifold exposure to the live forms and thyroid gland being an important organ of the body in maintaining the general metabolism, any deformity or malfunctioning in the same may result into various toxicological effects on the overall physiology costing a high price to the body. Thus an investigation of the effects of

acrylamide on the thyroid gland becomes vital. In this study, emphasis was placed on the monitoring of the thyroid hormone levels and hematological values in the plasma collected from the experimental animals along with mortality, body weight and visual morphological changes.

Materials and Methods

To achieve the aim of the investigation the following materials and methods were carried out. Acrylamide monomer of (99%) purity, was obtained from central Drug House (P) Ltd. Bombay, in powder form. It was dissolved in double distilled water to obtain 1 % solution of acrylamide monomer and then was given at 3 dose levels below LD_{50}. *Mus musculus* (Swiss albino mice) were used for the present investigation. They were fed on standard mice feed obtained from Hindustan lever Ltd. New Delhi and water *ad libitum*. Healthy Swiss albino mice each of 30 ± 2 gms. were selected for the present investigation. The experiment was divided in 3 groups each with 10 animals

I. Experimental Group E_1

II. Experimental Group E_2

III. Experimental Group E_3

A 'Control Group' along with each experimental group was also maintained with the same number of animals further divided as C_1, C_2, C_3. The experimental Groups E_1, E_2, E_3 were given acrylamide with 3 different dose levels : 5 mg/kg body wt. of acrylamide, 15 mg/kg body wt. of acrylamide, 25 mg/kg body wt. of acrylamide and the control groups C_1, C_2, C_3 were given equal volumes of double distilled water equivalent to the volume of the chemical given to its respective experimental groups. The dosing of all the groups was carried out orally (po) with a gavage needle.

The duration of the experiment was 60 days, all the doses were given on alternate days. During the complete duration of the experiments, a total of 30 doses were given to each animal in all the experimental and control groups. Animals were autopsied after 24 hrs. of oral administration of the last dose on 61^{th} day. They were scarified by cervical dislocation. Blood was collected by cardiac puncture and stored in heparinized plastic tubes and placed on ice until other samples were collected from all the animals. Plasma was collected by spinning the blood samples at 5000 rpm for 10 minutes.

Animals of all the control and experimental groups were checked daily for mortality. Initial and final body weights of experimental and control animals were taken and recorded and the differences in the body weight gain were calculated. Mice were observed and examined twice each day (9.00 A.M. and 5.00 P.M.) for any deformities, visual morphological symptoms like bleeding, hair loss, salivation, irritations, tremors, limb deformities, splaying of hind limbs or appearance of tumours were noted and recorded in all the animals. The plasma samples were analyzed for tri-iodothyronine (T_3), Thyroxine/tetraiodothyronine (T_4) and Thyroid Stimulating Hormone (TSH) using commercially available radio immuno assay (RIA) kits (Syntron Bioresearch, Inc). The procedures outlined in the kit were followed and results were calculated. Blood collected was tested for haematological indices like haemoglobin content, erythrocyte count and haematocrit value.

Observations and Results

The mortality was observed to be nil in all the experimental groups (Table 1). The control animals gained an average body weight while there was a significant reduction in the body weight gains of all the experimental groups and the sequence was $E_1 < E_2 < E_3$ (Table 2). Toxicological symptoms like tremors, lesions, bleeding, spots of blood and cuts on skin along with general hair loss from the body, especially on the face region were found in the mice of almost all the groups. Weakness in

Table 1 : Mortality rate

Experimental group	Total number of animals	Number of dead animals	Survival time (days)	Mortality rate in (%)
Control –C	10	0	60	0.00%
Group – E_1	10	0	60	0.00%
Group – E_2	10	0	60	0.00%
Group – E_3	10	0	60	0.00%

C = Control; E = Experimental

Table 2 : Mean ± SEM Body weight

Group	Initial body weight (grams)	Final body weight (grams)	Difference in body weight (grams)	Reductions in body weight gain (grams)
C_1	30.44±0.854	35.01±0.148	4.57±0.456	1.77
E_1	30.23±0.172	33.03±0.315#	2.80±0.388#	
C_2	30.39±0.182	35.01±0.105	4.62±0.244	1.69
E_2	30.22±0.341	32.15±0.583#	2.93±0.575#	
C_3	30.23±0.044	35.23±0.838	5.00±0.368	4.05
E_3	30.30±0.014	31.25±0.627	1.95±0.622#	

Mean value ± SE; C = Control; E = Experimental; P>0.05 not significant; #P<0.05 significant

limbs was prominent affecting the movements of the animals and the general response and activities along with the feeding and drinking behaviour was also found to be reduced. The plasma levels of T_3 and T_4 showed a marked decrease in the experimental groups than the controls in the sequence $E_1 < E_2 < E_3$ while a constant increase in the levels of TSH was noted in the sequence $E_1 < E_2 < E_3$ among the experimental groups (Table 3). The values of haematological parameters like haemoglobin content, erythrocyte count and haematocrit were found to be reduced in the order $E_1 < E_2 < E_3$ (Table 4).

Discussion and Conclusion

Acrylamide has emerged as a widely used chemical which has invaded our daily life activities. An important fact revealed regarding acrylamide is that it is quite safe as the polymeric form but has proven to be harmful in the monomeric form. Reports by Crofton *et. al.* (1996), Kuperman (1958), Mc Collister *et. al.* (1964), Sobel *et al.* (1986), Tilson (1981), Dearfild *et. al.* (1988) and Friedman *et. al.* (1995) confirm the multiple toxicities exerted by this chemical. The exposure to acrylamide can be through various pathways– direct through ingestion, absorption and inhalation or indirect by consumption of products which incorporate the use of the same in their processings. The toxicity levels of any chemical depends upon the route, rate of absorption, concentration and the duration of the exposure of the same in different species (Marty 1998; Sumner *et. al.*, 2001).

Various toxicological effects observed in the animals of the experiment direct us to focus on a deep study and evaluate the possible causes regarding this toxicity of the chemical.

The concentration of the dose administered might not have been sufficient to

Table 3 : Mean ± SEM Thyroid Hormone Levels

Groups	T3 (µg/ml)	T4 (µg/ml)	TSH (µIU/ml)
C_1	1.80±0.185	10.95±0.589	2.68±0.345
E_1	1.17±0.082	9.12±0.712	3.12±0.424
C_2	1.72±0.060	15.12±0.371	2.68±0.314
E_2	0.71±0.625#	7.18±0.306#	3.87±0.435#
C_3	1.80±0.206	15.16±0.355	2.86±0.195
E_3	0.49±0.310#	5.26±0.433#	4.14±0.374#

Mean value ± SE; Mean value ± SE; C = Control; E = Experimental; P>0.05 not significant; #P<0.05 significant

Table 4 : Mean ± SEM Haematological Parameters

Groups	Hb (%) g/ml	RBC count (million/mm^3)	PCV (%)
C_1	15.42±0.406	9.41±0.308	42.53±0.610
E_1	13.06±0.263#	6.96±0.211#	34.94±0.645#
C_2	15.12±1.364	9.44±0.489	41.94±0.504
E_2	9.18±0.306#	4.20±0.627#	30.77±0.971#
C_3	15.16±0.300	9.70±0.555	41.83±0.419
E_3	7.86±0.603#	3.58±0.602#	26.65±0.807#

Mean value ± SE; Mean value ± SE; C = Control; E = Experimental; P>0.05 not significant; #P<0.05 significant

cause lethality, but definitely disturbed the BMR due to disfunctioning of the thyroid gland and affecting its metabolism, which was clearly evident as the decreased mean body weight gains of the experimental animals. The decreased feed and water consumption could be attributed to the sluggish movements, muscular and nervous weakness along with alterations in hunger and thirst centers of the brain due to the nuerotoxicity which the chemical is known to cause (Le quesne, 1980). The sighted toxicological symptoms like redness, cuts and lesions on the skin along with hair loss from the body can be accredited to the property of acrylamide deposition in the skin after 28 hrs. of administration thereby causing an acute extra vascular haemolysis, along with damage to the hair follicles.

The deflections in the various haematological parameters of the experimental animals as compared with that of controls lead us to investigate the possibilities responsible for the same. Low levels of haemoglobin might be either due to the retarded synthesis or destruction. Since the standard mice feed given to the animals contained all the balanced levels of iron, folic acid and vitamin B_{12} which are essential for the synthesis of hemoglobin, this possibility gets ruled out and chances of destruction of haemoglobin brightens high. As reported by Hashimoto and Aldri (1970), Bergmark *et al.* (1993) and Barber *et al.* (2001) that acrylamide is electrophilic and covalently binds to the cysteine residues and forms adducts with sulphydral groups on haemoglobin resulting in the loss of haem part

of haemoglobin molecules thereby reducing the amount of haemoglobin in blood, which in turn may also be responsible for the anaemic conditions as evident by the low levels of RBC counts in the experiment.

The present study exhibited changes in the levels of the thyroid hormones. Since the thyroid hormones directly control the growth and development of tissues, cell respiration, total energy expenditure along with turn over of essentially all substrates, vitamins and hormones including thyroid hormones itself therefore the various toxic symptoms like low body weight gain, sluggish appearance, bizarre behaviour, bulging of eyes along with diminished consumption of food and water can be synchronized with the increased levels of TSH and consequent reduced levels of T_3 and T_4.

Acrylamide has thus influenced almost every aspect of the body functions and has disorganized the complete physiology of the experimental animals. The usage and consumption of this chemical in our day to day life is furthur facilitating its swift entry in the food chain affecting every trophic level and thus substantiating a serious threat not only for man but for all the live forms on the earth. Therefore, an expanded, corollary and descriptive study at the molecular level including the methods of immunochemistry, thermokinetics and thermodynamics for the specific loci determination of the disorganization of blood as well as thyroid gland is needed to be explored.

It would furnish the scientists with better options which would help them to search for a median path regarding the use of this chemical and take preventive measures to save the living beings from the hidden disasters of this chemical.

Acknowledgement

The authors are indebted to Prof. A.L. Bhatia for his constructive criticism and reviewing the paper and the Head and the Department of Zoology for providing facilities to carry out this research work.

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