Toxicology Reports 3 (2016) 673–678

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Lack of adverse health effects following 30-weeks of dietary exposure to acrylamide at low doses in male F344 rats

Jayadev Raju[a,*], Andrea Kocmarek[a], Jennifer Roberts[a], Marnie Taylor[a], Dominique Patry[b], Emily Chomyshyn[b], Don Caldwell[b], Gerard Cooke[a], Rekha Mehta[a]

[a] Regulatory Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch Health Canada, Ottawa, Ontario, Canada

[b] Scientific Services Division, Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch Health Canada, Ottawa, Ontario, Canada

ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form 29 August 2016
Accepted 30 August 2016
Available online 31 August 2016

Keywords:
Acrylamide
Clinical biochemistry
Diet
F344 rats
Hematology
Pathology
Testosterone
Toxicity

ABSTRACT

Understanding the health hazards following exposure to food-borne acrylamide, especially at low levels typified by human diets, is an ongoing food safety issue. We recently published results from a study that aimed to understand the effects of acrylamide short-term exposure at doses known to cause tumors in rodents, demonstrating that a number of key toxicological end points were altered by acrylamide exposure. Additionally, we reported that at much lower doses for 30 weeks of exposure, dietary acrylamide was 'not a complete carcinogen' to the colon in an organ-specific rodent carcinogenesis study but acted as a co-carcinogen along with azoxymethane (AOM, a colon-specific carcinogen). Here, we present toxicological data from a sub-set of this long-term exposure study from animals that received saline (instead of AOM). Briefly, male F344 rats were randomized to receive acrylamide at 0.5, 1.0 and 2.0 mg/kg diet (~0.02, 0.04, and 0.09 mg/kg BW/day, respectively) or no acrylamide (control), for 30 weeks; all rats were then euthanized and their tissues harvested and processed for toxicological evaluation. We report that at the doses tested, acrylamide did not cause any changes in general well-being, body weight or food intake. Similarly, acrylamide did not cause any biologically relevant change in parameters associated with immunophenotyping, serum biochemistry or hematology. Histopathology assessment of tissues showed no changes except in the testis, where non-specific mild lesions were observed in all the groups, inclusive of the controls. No neuropathological effects of acrylamide were observed in the brain and nerve tissues. Together, these results suggest that acrylamide administered to rats through the diet at low doses for 30 weeks did not cause any toxicologically relevant changes. Given that the doses of acrylamide in the current study are low and are comparable to human dietary exposure, this null-effect study provides data that contribute to the body of scientific evidence relevant to understanding the health effects of acrylamide.

1. Introduction

Acrylamide, a known rodent carcinogen, was discovered in human food by the Swedish National Food Agency (2002) [29] and since then acrylamide has been of concern in the context of food safety. Acrylamide is found in a variety of popular foods including baby food, coffee, cereal, French fries, etc., at concentrations that may exceed 2 mg/kg of food [32,13,20]. At high doses, including those known to induce rodent tumors (2-year cancer bioassay), acrylamide has been shown to cause other non-cancer toxic effects, including neurotoxicity and reproductive toxicity [15,19,26]. The toxicokinetics and toxicodynamics of acrylamide have been thoroughly studied [26]. Previous studies have examined the oral toxicity of acrylamide exposure via the three major routes—food, drinking water and gavage using relevant rodent models [19,5,6,17,11,3]. Acrylamide has been detected in multiple tissues of orally-exposed rodents, including the brain, heart, liver, lung, spleen, kidney, thymus, pancreas, and blood [1]; it has been found in human breast milk and can cross the placental barrier [27]. Once inside the body acrylamide can form adducts with hemoglobin, be conjugated with glutathione, or is oxidized by CYP2E1 to form the more reactive epoxide glycidamide [8].

* Corresponding author at: Regulatory Toxicology Research Division, Bureau of Chemical Safety, Food Directorate, Health Canada, C209 Frederick G. Banting Research Centre, Postal Locator 2202C, 251 Sir Frederick Banting Driveway, Ottawa, K1A 0K9, Ontario, Canada.
E-mail address: jayadev.raju@hc-sc.gc.ca (J. Raju).

http://dx.doi.org/10.1016/j.toxrep.2016.08.010

Most rodent studies have focused on acrylamide at toxicologically relevant high doses, which are known to cause tumors [19,5,6,17,11,3]. We recently published two *in vivo* studies using the azoxymethane (AOM)-induced rat colon carcinogenesis model, demonstrating that food-borne acrylamide at high doses and exposed for 8 weeks did not augment colon precancerous lesions [22]; and at low doses and exposed for 30 weeks was 'not a complete carcinogen' in the colon [21]. The latter study is one of the few that examined food-borne acrylamide exposure, at levels similar to those found in human foods in a long-term rodent model. In the 30-week carcinogenesis study, the highest dose of acrylamide (2 mg/kg diet) increased the size of colon tumors in AOM-injected rats and resulted in a higher occurrence of adenocarcinoma-type lesions, suggesting that acrylamide may act as a co-carcinogen in the colon and exacerbate the effects of AOM [21]. In the same paper, we reported that dietary acrylamide did not aid in the progression of established human colon tumor xenografts in the nude (*nu/nu*) mouse model, and hence may not play a role in colon tumor promotion [21]. The aim of the present study was to understand if dietary acrylamide caused non-cancerous and non-linear toxic effects in male F344 rats exposed to low doses similar to human exposure levels. Briefly, a set of rats randomized to four acrylamide dose groups (0, 0.5, 1.0, and 2.0 mg/kg diet) received saline (vehicle) instead of AOM, and were examined for pathology, hematology and clinical biochemistry after 30-weeks of dietary exposure. We report that acrylamide administered to rats through the diet at low doses for 30 weeks did not cause any toxicologically relevant changes.

2. Methods

2.1. Animal experimentation, husbandry and diets

The animal experimentation in this study was approved by the Health Canada Ottawa Animal Care Committee. The animals in this study were a complete sub-set from our earlier carcinogenesis study that served as a saline-treated control group [21]. All animals were cared for according to the guidelines established by the Canadian Council on Animal Care. Briefly, six-week-old male F344 rats were obtained from Charles River Laboratories (St. Constant, Quebec, Canada) and were pair housed with a 12 h:12 h light-dark cycle, and with relative humidity and temperature controlled at 55% and 22 °C, respectively. The rats had *ad libitum* access to water and either basal diet (during the acclimatization phase) or experimental diet (during the experimental phase). Diets were based on the AIN-93G semi-synthetic formula modified to contain 7% corn oil [2] and obtained from Research Diets, Inc. (New Brunswick, New Jersey, USA). A week after acclimatization, rats were randomized ($n = 8$/group) to experimental diets containing acrylamide (Cat. A9099, purity ≥ 99%; Sigma-Aldrich Canada Co., Oakville, Ontario, Canada) at levels of 0.5, 1.0, or 2.0 mg/kg diet and the basal diet with no acrylamide that served as the control. Acrylamide was added to the powdered diets using a Hobart mixer and pelleted in our diet preparation facility. During pelleting, the temperature was maintained below 35 °C to avoid any additional acrylamide formation during the processing of the diets. Diets were stored at 4 °C, in the dark, until use. The homogeneity, stability and background levels of acrylamide in rodent diets have been earlier reported by the National Toxicology Program (NTP; 2012) [19], where the levels of acrylamide analyzed by capillary gas chromatography with flame ionization detection, were found to be below the Limit of Quantitation in all their control diets. Our diet preparations were similar to that of the rodent diets utilized in the NTP studies [19], and based on our diet preparation conditions, any background acrylamide levels in our diets are expected to be negligible and below the Limit of Quantitation.

Food consumption by the rats was measured biweekly and diets were replaced weekly. The health and general behaviour of the rats were monitored daily and body weights were measured twice a week. After the 30-week experimental period, rats were killed by exsanguination under isoflurane anesthesia; organs were dissected and their wet weights recorded. Collected organs included the brain, tibial nerve, liver, kidney, pancreas, epididymis, heart, mesenteric lymph node, adrenal glands, colon, small intestine, thymus, spleen, prostate, lungs, and testes. The organs were fixed in neutral buffered formalin, except the testes which were fixed in modified Bouin's solution, for pathological examination. Blood to be used for serum biochemistry was collected in BD Vacutainer SST™ blood collection tubes (Becton-Dickinson, Franklin Lakes, New Jersey, USA) and allowed to clot at room temperature. Serum was separated by centrifugation at $700 \times g$ and stored at −80 °C until analysis. Blood to be used for hematology and immunophenotyping was collected in BD Vacutainer K_2-EDTA blood collection tubes.

2.2. Histopathology

The tissue sections were assigned to coded ID numbers to ensure the pathologist (D.C.) was blinded to treatment groups. Microscopic examinations were conducted on formalin-fixed (modified Bouin's solution for testes), paraffin-embedded 5-μm sections of the lung, liver, kidney, pancreas, epididymis, heart, mesenteric lymph nodes, colon, small intestine, adrenal gland, thymus, spleen, prostate and testis stained with hematoxylin and eosin (H&E); the brain and tibial nerve sections were stained with hematoxylin-phloxine-saffron (HPS).

2.3. Hematology and serum clinical biochemistry

An AcT5 Diff CP analyzer (Beckman Coulter Canada, Inc., Mississauga, Ontario, Canada) was used for the plasma analysis of the following hematological parameters: red blood cell count, hematocrit, hemoglobin (HGB), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet counts, mean platelet volume, and white blood cell counts (total and differentiated). A Pentra 400 (HORIBA ABX Inc., Irvine, California, USA) was used to measure serum clinical biochemistry parameters including amylase, albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), calcium, carbon dioxide (CO_2), chloride, cholesterol, including high and low density lipoprotein cholesterol, creatinine, creatine kinase (CK), glucose, iron, lipase, magnesium, phosphorous, potassium, sodium, sodium: potassium ratio, total protein, triglycerides, and blood urea nitrogen (BUN).

2.4. Serum testosterone

Serum testosterone (T) assays were conducted on all males in duplicate using a commercial ELISA kit, (IBL America, Minneapolis, Minnesota, USA). The cross reactivity of dihydrotestosterone (DHT) is quoted as 0.8%. The inter-and intra-assay coefficients of variation (CV%) for serum T were 3.3% ($n = 3$) and 7.1% ($n = 3$), respectively. Intra- and inter-assay coefficients of variations (CV%) for ELISA assays were experimentally derived from the data provided with the PolarStar Optima plate reader (BMG Labtech, Ortenberg, Germany).

2.5. Immunophenotyping

Leukocytes in whole blood and the thymus were characterized. Briefly, red blood cells were lysed in the whole blood samples. Thy-

mus tissues were gently crushed in a 70 μm nylon cell strainer (BD Falcon, Bedford, MA, USA) and rinsed into a 50 mL tube in Dulbecco's phosphate buffered saline (Gibco/Invitrogen, Grand Island, New York, USA) to create thymocyte suspensions. Thymocyte and blood suspensions were standardized to ensure equal cell concentrations per mL of suspension. Mouse anti-rat monoclonal antibodies from BD Biosciences (Mississauga, Ontario, Canada) were used to characterize T lymphocytes, helper T cells, cytotoxic T cells, B cells, and natural killer cells in the whole blood and thymus. Monocytes were also measured in whole blood. Suspensions were mixed with respective antibodies and were fixed with 2% formaldehyde solution. Lymphocyte subset analyses were conducted by flow cytometry on a BD FACSCaliber System (BD Biosciences, Mississauga, Ontario, Canada).

2.6. Statistical analysis

Serum testosterone levels were compared using ANOVA and a post-test for linear regression using Prism Version 6.01 (GraphPad Software, Inc., La Jolla, California, USA). All other data were analyzed using ANOVA using SigmaStat$^{(R)}$ 3.1 software (Systat Software Inc., Point Richmond, California, USA). $P < 0.05$ was considered significant for all statistical tests.

3. Results and discussion

Acrylamide, a known rodent carcinogen, genotoxin and neurotoxin, is formed as a consequence of a Maillard reaction between the carbonyl group of reducing sugars and asparagine in a range of human foods processed at high temperature [28]. Acrylamide is classified as a 'probable human carcinogen—Class 2A' [15], and there is ongoing research to understand its health effects for hazard identification and regulatory risk assessments (reviewed in [10]). Earlier, we demonstrated that food-borne acrylamide at levels comparable to those found in human foods was 'not an independent carcinogen to the colon' in male F344 rats using the AOM-induced colon carcinogenesis model [21]. In that study, the carcinogen-treated rats were dosed with 0.5, 1.0, and 2.0 mg/kg diet for 30 weeks and the average intake of acrylamide was calculated as 0.018, 0.036 and 0.072 mg/kg BW/day, respectively [21]. Risk assessments typically examine the effects of high doses of chemicals to determine lowest/no observed adverse effect levels (L/NOAELs). Emphasizing the importance of low dose effects in toxicology [4], addressed the "low-dose hypothesis" which postulates that low doses of chemicals can have effects with nonmonotonic dose-response curves leading to non-linear responses that would not necessarily be predicted from their effects at high doses. Here, we present data from the previous study [21], pertaining to our hematological, clinical biochemistry and pathological findings from a sub-set of rats that were exposed to low-dose dietary acrylamide but not AOM, the carcinogen.

In the current study, none of the rats exhibited signs of discomfort or changes in their general behaviour that could be associated with acrylamide-toxicity. Prior to the commencement of the treatments, the body weight of rats ranged from 113 to 190 g and the weekly measured body weights did not differ between dietary groups at any given time point (Table 1). At the end of the 30-week exposure (at necropsy), there were no acrylamide-related change in body weights. It should be noted that in the study reported by [19], the lowest dose of acrylamide at a dose of 0.33 mg/kg BW/day caused no change in body weight at 28 and 32 weeks. Relative wet weights of the thymus, heart, liver, spleen, pancreas, adrenal glands, testis and epididymis were calculated as g per 100 g BW and were not significantly different by comparison to the control (Table 2). Food consumption was calculated as g of food per kg BW per day (Table 3), with no significant difference observed between the acrylamide-treated groups and the control. Based on the calculated food consumption, the mean overall intake of acrylamide in the 0.5, 1.0 and 2.0 mg/kg diet dose groups was calculated as 0.022 ± 0.001, 0.044 ± 0.002, and 0.088 ± 0.004 mg/kg BW/day, respectively. We employed allometric scaling to assess the human equivalent dose (HED) of acrylamide from the dietary exposure the rats received, by using the exponent 0.67 for body surface area [31,24]. We calculated the HED as ~0.003, 0.007 or 0.014 mg/kg/BW for rats exposed to acrylamide at 0.5, 1.0 or 2.0 mg/kg diet. These levels are congruent with the actual upper human exposure levels of 0.003–0.0034 mg/kg BW/day (90–95th percentile of high level human consumers) detected by different food regulatory agencies [33,14,9,34]. In addition, based on national and regional estimates, a dietary exposure to acrylamide of 0.001 mg/kg BW per day was representative of the mean for the general population (including children), and a dietary exposure of 0.004 mg/kg BW per day represented consumers with a high dietary exposure [16].

H&E stained sections of liver, kidney, pancreas, epididymis, heart, mesenteric lymph node, adrenal glands, thymus, spleen, prostate and lung were examined and no neoplastic or non-neoplastic lesions were found in either the acrylamide-treated or control rats. Acrylamide-induced testicular toxicity has been widely studied; acrylamide, at toxicologically relevant oral doses (2.5–50 mg/kg BW/day) for 14 days, caused significant effects on Leydig and germ cells in the testes of male F344 rats [7]. In the current study, we did not observe such pathological changes; however, in the testis, mild degeneration (vacuolation) of the seminiferous epithelia with single or few atrophied tubules were noted in all the rats (including the controls) with no evidence for an acrylamide-related effect. These non-specific mild testicular tubule atrophies are likely related to the F344 strain and age (>36 weeks) as observed at necropsy by [12]. Additionally, there is historical evidence of mild testicular tubule atrophies in control male F344 rats (27%) as reported in the 2-year drinking water study of acrylamide by the [19]. It should be noted that in a previous oral exposure study, acrylamide at a high dose of 40 ppm (equivalent to 4.4 mg/kg BW/day) administered for 12 weeks caused testicular toxicity, but no effects

Table 1
Mean body weight (g) of male F344 rats in a 30-week feeding study of acrylamide[a].

Dose	Body weight (g) (Relative percent in comparison to Control)						
	Week 1	Week 5	Week 10	Week 15	Week 20	Week 25	Week 30
Control	186.4 ± 8.0	279.2 ± 9.1	351.5 ± 9.9	395.4 ± 9.5	423.0 ± 8.6	447.4 ± 8.7	458.1 ± 10.7
0.5 mg/kg	211.0 ± 3.9 (113)	307.7 ± 4.3 (110)	380.8 ± 5.7 (108)	420.7 ± 6.1 (106)	449.7 ± 6.2 (106)	473.5 ± 6.5 (106)	486.6 ± 8.9 (106)
1.0 mg/kg	197.8 ± 4.6 (106)	290.0 ± 5.5 (104)	363.4 ± 7.3 (103)	407.4 ± 8.6 (103)	434.8 ± 10.2 (103)	459.2 ± 10.3 (103)	466.7 ± 10.4 (102)
2.0 mg/kg	201.1 ± 4.8 (108)	297.7 ± 5.3 (106)	365.2 ± 5.4 (104)	405.0 ± 5.7 (102)	435.7 ± 5.9 (103)	455.1 ± 5.3 (102)	473.3 ± 6.2 (103)

[a] Weights expressed as mean ± standard error of the mean.

Table 2
Mean organ wet weights[a] (g/100 g BW) of male F344 rats in a 30-week feeding study of acrylamide.

	Organ weights (g/100 g BW)			
	Control	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg
Thymus	0.0338 ± 0.0024	0.0361 ± 0.0016	0.0346 ± 0.0022	0.0400 ± 0.0031
Heart	0.2564 ± 0.0060	0.2495 ± 0.0053	0.2621 ± 0.0043	0.2636 ± 0.0031
Liver	3.1436 ± 0.0356	3.1858 ± 0.0549	3.1278 ± 0.0301	3.0849 ± 0.0636
Spleen	0.1733 ± 0.0027	0.1822 ± 0.0041	0.1798 ± 0.0031	0.1809 ± 0.0022
Pancreas	0.1989 ± 0.0077	0.2098 ± 0.0056	0.2205 ± 0.0087	0.2231 ± 0.0090
AG[b]	0.0102 ± 0.0003	0.0099 ± 0.0003	0.0102 ± 0.0003	0.0103 ± 0.0002
Prostate	0.0844 ± 0.0071	0.0692 ± 0.0037	0.0643 ± 0.0050	0.0698 ± 0.0055
Testis	0.7442 ± 0.0177	0.7103 ± 0.0373	0.7937 ± 0.0257	0.7799 ± 0.0303
Epididymis	0.2028 ± 0.0064	0.1894 ± 0.0121	0.2097 ± 0.0029	0.2001 ± 0.0071

[a] Weights expressed as mean wet weights per 100 g body weight ± standard error of the mean. [b] AG = adrenal glands.

Table 3
Food and acrylamide consumption of male F344 rats in a 30-week feeding study of acrylamide.

Dose	Mean food consumption[a] (g/kg BW/day)			(Relative percent in comparison to Control)			
	Week 1	Week 5	Week 10	Week 15	Week 20	Week 25	Week 30
Control	81.1 ± 2.5	55.8 ± 1.3	46.7 ± 1.3	41.2 ± 1.1	39.5 ± 0.7	37.9 ± 0.6	34.5 ± 0.6
0.5 mg/kg	78.9 ± 1.0 (97)	53.9 ± 0.7 (97)	44.6 ± 0.9 (95)	39.4 ± 0.6 (96)	39.4 ± 0.7 (100)	35.9 ± 0.5 (95)	33.9 ± 0.4 (98)
1.0 mg/kg	78.7 ± 1.3 (97)	53.5 ± 0.7 (96)	44.6 ± 0.6 (95)	39.7 ± 0.6 (96)	40.0 ± 1.2 (101)	36.3 ± 0.6 (96)	33.6 ± 0.5 (97)
2.0 mg/kg	79.7 ± 1.3 (98)	54.9 ± 0.9 (98)	43.9 ± 0.9 (94)	40.0 ± 0.5 (97)	39.8 ± 0.5 (101)	36.9 ± 0.4 (97)	33.9 ± 0.4 (98)

Dose	Mean acrylamide consumption (μg/kg BW/day)						
	Week 1	Week 5	Week 10	Week 15	Week 20	Week 25	Week 30
Control	0	0	0	0	0	0	0
0.5 mg/kg	39.5 ± 0.5	26.9 ± 0.4	22.3 ± 0.5	19.7 ± 0.3	19.7 ± 0.4	17.9 ± 0.2	17.0 ± 0.2
1.0 mg/kg	78.7 ± 1.3	53.5 ± 0.7	44.6 ± 0.6	39.7 ± 0.6	40.0 ± 1.2	36.3 ± 0.6	33.6 ± 0.5
2.0 mg/kg	159.4 ± 2.5	109.7 ± 1.8	87.9 ± 1.9	79.9 ± 1.1	79.6 ± 1.1	73.9 ± 0.8	67.8 ± 0.8

[a] Food consumption expressed as mean food consumed per kg body weight of rat per day ± standard error of the mean. Food was measured by cage, divided by number of days since previous measurement, divided by 2 rats per cage, divided by mean body weight of the 2 rats.

Table 4
Serum clinical biochemistry of male rats in a 30-week feeding study of acrylamide.

	Control	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg
Albumin (g/L)	32.0 ± 1.0	32.8 ± 0.7	32.4 ± 0.7	32.4 ± 0.7
ALT (U/L)	59.5 ± 6.6	55.1 ± 3.9	76.3 ± 25.9	58.5 ± 4.6
ALP (U/L)	125.3 ± 8.3	120.6 ± 3.4	149.2 ± 18.7	126.9 ± 5.7
Amylase (U/L)	3112 ± 63	3163 ± 89	3034 ± 109	2931 ± 59
AST (U/L)	81.3 ± 4.7	79.3 ± 5.0	80.8 ± 9.2	77.4 ± 4.2
BUN (mmol/L)	6.3 ± 0.3	6.3 ± 0.1	5.9 ± 0.2	6.1 ± 0.2
Calcium (mmol/L)	2.95 ± 0.11	3.00 ± 0.08	2.99 ± 0.08	2.99 ± 0.09
Chloride (mmol/L)	98.6 ± 0.8	98.4 ± 0.9	98.5 ± 0.9	98.6 ± 0.9
Cholesterol (mmol/L)	3.1 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	3.3 ± 0.1
HDL (mmol/L)	0.86 ± 0.05	0.88 ± 0.03	0.83 ± 0.03	0.87 ± 0.04
LDL (mmol/L)	0.29 ± 0.01	0.31 ± 0.02	0.30 ± 0.01	0.30 ± 0.02
CK (U/L)	448.7 ± 28.0	388.2 ± 30.1	340.1 ± 31.6	309.2 ± 38.0*
CO_2 (mmol/L)	29.5 ± 0.3	29.7 ± 0.2	30.4 ± 0.2	31.0 ± 0.3*
Creatinine (mmol/L)	30 ± 3	30 ± 3	32 ± 3	29 ± 2
Glucose (mmol/L)	8.2 ± 0.5	8.2 ± 0.4	9.2 ± 0.6	9.6 ± 0.6
Iron (μmol/L)	33.55 ± 0.72	33.01 ± 0.33	31.40 ± 0.71	31.12 ± 0.92
Lipase (U/L)	6.8 ± 0.6	6.7 ± 0.8	6.7 ± 0.9	8.2 ± 0.8
Magnesium (mmol/L)	0.59 ± 0.03	0.61 ± 0.02	0.61 ± 0.01	0.62 ± 0.02
Phosphorus (mmol/L)	1.8 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Potassium (mmol/L)	4.1 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.1
Sodium (mmol/L)	140.8 ± 1.0	140.9 ± 0.8	140.7 ± 0.7	140.8 ± 0.8
Total Protein (g/L)	68.6 ± 1.7	70.2 ± 0.6	68.8 ± 0.2	69.5 ± 0.9
Triglycerides (mmol/L)	3.17 ± 0.17	3.65 ± 0.28	3.61 ± 0.25	4.05 ± 0.27
Testosterone (ng/mL)	1.791 ± 0.325	2.019 ± 0.205	2.390 ± 0.227	2.534 ± 0.417

An asterisk (*) denotes value that is significantly different ($p < 0.05$) from the control (no acrylamide exposure). ALT—alanine transaminase, ALP—alkaline phosphatase, AST—aspartate transaminase, BUN—blood urea nitrogen, HDL—high density lipoprotein, LDL—low density lipoprotein, CK—creatine kinase, CO_2—carbon dioxide.

were observed at either 10 or 20 ppm [30]. Supporting our data related to the testes, we found no changes in serum testosterone levels between the control and any of the acrylamide-treated groups (Table 4), suggesting no male hormone-specific testicular toxicity at low dose exposure. Similarly [7], reported changes in serum testosterone in male F344 rats exposed at a dose of 10 mg/kg BW/day acrylamide or greater, with no changes in animals tested at a lower dose of 2.5 mg/kg BW/day. To assess any neurological

Table 5
Hematology of male F344 rats in a 30-week feeding study of acrylamide.

	Control	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg
WBC	4.2 ± 0.3	4.6 ± 0.2	4.3 ± 0.1	4.1 ± 0.1
RBC	8.57 ± 0.06	8.78 ± 0.08	8.70 ± 0.05	8.67 ± 0.06
HGB	142 ± 1	146 ± 1*	144 ± 1	145 ± 1*
HCT	0.414 ± 0.003	0.424 ± 0.003	0.421 ± 0.002	0.422 ± 0.002
MCV	48 ± 0	48 ± 0	48 ± 0	49 ± 0
MCH	16.6 ± 0.1	16.6 ± 0.1	16.6 ± 0.1	16.7 ± 0.1
MCHC	343 ± 1	344 ± 1	343 ± 1	345 ± 1
RDW	12.9 ± 0.1	12.7 ± 0.1	12.6 ± 0.1	12.7 ± 0.2
PLT	537 ± 8	540 ± 8	532 ± 8	517 ± 12
MPV	7.2 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.2
NE%	34.1 ± 1.4	35.8 ± 1.5	33.2 ± 1.1	31.1 ± 1.8
LY%	59.6 ± 1.4	58.2 ± 1.7	60.5 ± 0.9	62.2 ± 1.7
MO%	3.5 ± 0.3	3.1 ± 0.2	3.3 ± 0.4	3.5 ± 0.4
EO%	2.5 ± 0.1	2.5 ± 0.2	2.8 ± 0.2	2.9 ± 0.2
BA%	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
NE#	1.43 ± 0.13	1.66 ± 0.14	1.44 ± 0.06	1.27 ± 0.09
LY#	2.50 ± 0.17	2.65 ± 0.10	2.62 ± 0.08	2.53 ± 0.07
MO#	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02
EO#	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
BA#	0.01 0.00	0.02 0.00	0.01 0.00	0.02 0.00

An asterisk (*) denoted those that are significantly different ($p < 0.05$) from controls.
WBC—white blood cell, RBC – red blood cell, HGB—hemoglobin, HCT—hematocrit, MCV—mean corpuscular volume, MCH—mean corpuscular hemoglobin, MCHC—mean corpuscular hemoglobin concentration, RDW—red blood cell distribution width, PLT—platelet count, MPV—mean platelet volume, NE—Neutrophils, LY—Lymphocytes, MO—Monocytes, EO —Eosinophils, BA—Basophils.

Table 6
Leukocyte characterization of the thymus and whole blood of male rats in a 30-week feed study of acrylamide.

	Control	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg
Whole Blood				
T lymphocytes[a] CD3+	48.3 ± 2.4	50.2 ± 2.7	53.4 ± 2.4	52.2 ± 3.8
Helper T cells[a] CD3+/CD4+	30.6 ± 1.9	30.9 ± 1.8	32.7 ± 1.8	31.4 ± 2.6
Cytotoxic T cells[a] CD3+/CD8a+	18.3 ± 0.9	20.2 ± 1.3	21.7 ± 0.8	21.8 ± 1.5
B cells[a] CD45RA+/CD3-	13.9 ± 1.8	14.1 ± 1.9	15.7 ± 2.0	16.2 ± 1.5
NK[b] cells[a]	2.4 ± 0.2	2.9 ± 0.3	3.5 ± 0.5	2.8 ± 0.2
CD161a+ (high)/CD3-CD161a+ (med)/CD3-	30.9 ± 2.8	29.8 ± 4.3	24.7 ± 4.7	26.0 ± 5.2
Monocytes[a] CD4+/CD3-	7.2 ± 0.9	6.1 ± 0.3	7.0 ± 0.4	7.2 ± 0.5
Thymus				
Immature thymocytes[a] CD4-/CD8-	4.03 ± 0.29	3.92 ± 0.21	3.81 ± 0.20	4.15 ± 0.22
Immature thymocytes[a] CD4+/CD8+	84.32 ± 0.57	84.32 ± 0.52	84.45 ± 0.33	84.37 ± 0.49
Helper T cells[a] CD4+/CD8-	8.60 ±0.41	8.69 ± 0.30	8.61 ± 0.21	8.59 ± 0.39
Cytotoxic T cells[a] CD4-/CD8+	3.07 ± 0.28	3.08 ± 0.28	3.11 ± 0.26	2.88 ± 0.13

[a] % leukocyte (CD45 +) cells.
[b] Natural Killer.

changes related to acrylamide exposure, cross-sections of the brain, at the level of the optic chiasm and another including brainstem and cerebellum, were examined and no lesions were found in any of the dietary groups.

A battery of tests was performed to determine the levels of biochemical parameters, some of which are used to assess liver and kidney function/damage (Table 4). Serum levels of the enzymes ALT, AST and ALP were not affected by acrylamide treatment. Total protein was unaffected between dietary groups, with no concomitant change in BUN values and creatinine. There was no change observed in the activity of serum CK in the 0.5 and 1.0 mg acrylamide/kg diet groups. However, a decrease ($P < 0.05$) was observed in the 2.0 mg acrylamide/kg diet group by comparison to the control (Table 4). This value still fell within the range considered biologically normal and thus is unlikely to indicate a toxic effect. Moreover, the BUN value, which is often taken in conjunction with the CK values to indicate renal dysfunction, was unaltered across dietary groups. Acrylamide is known to bind to CK leading to CK inactivation [25]; this may be a contributing factor to the decrease in serum CK activity that we have observed in rats from the 2.0 mg acrylamide/kg diet group.

An increase ($P < 0.05$) in serum CO_2 was observed in the highest tested acrylamide dose group of 2.0 mg/kg diet in comparison to the control; no changes were seen in the other two acrylamide dose groups (Table 4). The CO_2 levels in all the groups fell within the healthy range, indicating that the increase we observed in the 2.0 mg acrylamide/kg diet was not biologically relevant. Previously, we reported that acrylamide administered for a short duration (10

weeks) up to a dose of 50 mg/kg diet did not affect serum CO_2 levels in F344 rats [23].

In the hematological panel of tests (Table 5) and the leukocyte characterization (Table 6), we observed no changes in any parameters between control and acrylamide-treated groups, with the exception of HGB. An increase ($P < 0.05$) in HGB was observed in the 0.5 and 2.0 mg acrylamide/kg diet groups in comparison to the control. There was no dose-dependent pattern in the HGB levels, and no difference was observed between the 1.0 mg acrylamide/kg diet group and the controls (Table 5). The HGB values fell within the healthy range, and thus the increase in the upper and lower dose groups do not indicate toxicity. Other rodent studies reported that acrylamide in fact decreased hemoglobin levels [18,23], and the decrease was accompanied by other hematological changes, including decreased hematocrit and mean corpuscular hemoglobin [23] and increased bilirubin [18]. Such changes in associated hematological parameters were not observed in this study.

4. Conclusion

After a 30-week exposure, no toxicologically relevant changes were noted in male F344 rats fed acrylamide at levels of 0.5, 1.0, and 2.0 mg/kg diet, confirming the absence of any possible non-linear responses. These low dose levels were chosen as they are comparable to those found in a variety of foods commonly consumed by humans. Given that the acrylamide doses and exposure (diet and duration) strategies adopted in the current rodent study are comparable to human dietary exposure, the 'no-effect' data generated by this study will be useful in conjunction with other literature, and will contribute to the body of scientific evidence used to inform the risk assessment of dietary exposure to acrylamide.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Funding was provided by the Chemicals Management Plan, Government of Canada, Canada. A. Kocmarek is a recipient of a Visiting Fellowship to a Canadian Government Laboratory administered by Natural Sciences and Engineering Research Council, Canada. We would like to thank Ms. Emily Mutzke, Dr. Jason Fine and the staff of the animal holding facility for their excellent technical assistance.

References

[1] L. Abramsson-Zetterberg, J. Wong, N.G. Ilbäck, Acrylamide tissue distribution and genotoxic effects in a common viral infection in mice? Toxicology 211 (1–2) (2005) 70–76.
[2] American Institute of Nutrition, Report of the ad hoc committee on standards for nutrition studies, J. Nutr. 123 (1993) 1939–1951.
[3] F.A. Beland, P.W. Mellick, G.R. Olson, M.C. Mendoza, M.M. Marques, D.R. Doerge, Carcinogenicity of acrylamide in B6C3F(1) mice and F344/N rats from a 2-year drinking water exposure, Food Chem. Toxicol. 51 (2013) 149–159.
[4] L.S. Birnbaum, Environmental chemicals: evaluating low-dose effects, Environ. Health Perspect. 120 (2012) A143–A144.
[5] R.J. Bull, M. Robinson, R.D. Laurie, G.D. Stoner, E. Greisiger, J.R. Meier, J. Stober, Carcinogenic effects of acrylamide in Sencar and A/, J. Mice Cancer Res. 44 (1984) 107–111.
[6] R.J. Bull, M. Robinson, J.A. Stober, Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice, Cancer Lett. 24 (1984) 209–212.
[7] L. Camacho, J.R. Latendresse, L. Muskhelishvili, R. Patton, J.F. Bowyer, M. Thomas, D.R. Doerge, Effects of acrylamide exposure on serum hormones, gene expression, cell proliferation, and histopathology in male reproductive tissues of Fischer 344 rats, Toxicol. Lett. 211 (2) (2012) 135–143.
[8] E. Capuano, V. Fogliano, Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies, LWT—Food Sci. Technol. 44 (4) (2011) 793–810.
[9] European Food Safety Authority (EFSA), Scientific opinion on acrylamide in food EFSA panel on contaminants in the food chain (CONTAM), EFSA J. 13 (6) (2015) 321, http://dx.doi.org/10.2903/j.efsa.2015.4104 (4104).
[10] M. Friedman, Acrylamide: inhibition of formation in processed food and mitigation of toxicity in cells, animals, and humans, Food Funct. 6 (June (6)) (2015) 1752–1772, http://dx.doi.org/10.1039/c5fo00320b (PMID: 25989363).
[11] M.A. Friedman, L.H. Dulak, M.A. Stedham, A lifetime oncogenicity study in rats with acrylamide, Fundam. Appl. Toxicol. 27 (1995) 95–105.
[12] D.G. Goodman, J.M. Ward, R.A. Squire, K.C. Chu, Linhart MS neoplastic and nonneoplastic lesions in aging F344 rats, Toxicol. Appl. Pharmacol. 48 (April (2)) (1979) 237–248 (PMID: 473173 [PubMed – indexed for MEDLINE]).
[13] Health Canada, Acrylamide Levels in Selected Canadian Foods, 2013 http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/food-aliment/acrylamide/acrylamide_level-acrylamide_niveau_table-eng.php.
[14] Health Canada, Health Canada's Revised Exposure Assessment of Acrylamide in Food, 2012 http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/securit/chem-chim/food-aliment/acrylamide/rev-eval-exposure-exposition-eng.pdf.
[15] International Agency for Research on Cancer (IARC), IARC working group on the evaluation of carcinogenic risk to humans: some industrial chemicals IARC Monograms on the Evaluation of Carcinogen Risk to Humans, Vol. 60, IARC, Lyon, France, 1994.
[16] Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA). 2011. Evaluation of certain contaminants in food: seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series, 959: 9–21.
[17] K.A. Johnson, S.J. Gorzinski, K.M. Bodner, R.A. Campbell, C.H. Wolf, M.A. Friedman, R.W. Mast, Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats, Toxicol. Appl. Pharmacol. 85 (2) (1986) 154–168.
[18] R. Lal, M. Arora, A. Sharma, Acrylamide caused hematotoxicity on *mus musculus* through gavage, Indian J. Fundam. Appl. Life Sci. 1 (2011) 330–334.
[19] National Toxicology Program (NTP), 2012. NTP technical report on the toxicology and carcinogenesis studies of acrylamide (CAS No. 79-06-1) in F344/N rats and B6C3F1 mice (feed and drinking water studies). National Institutes of Health. Public Health Service. U.S. Department of Health and Human Services. NIH Publication No. 12–5917.
[20] D. Pacetti, E. Gil, N.G. Frega, L. Álvarez, P. Dueñas, A. Garzón, P. Lucci, Acrylamide levels in selected Colombian foods, Food Addit. Contam. Part B Surveillance 8 (2015) 99–105.
[21] J. Raju, J. Roberts, C. Sondagar, K. Kapal, S.A. Aziz, D. Caldwell, R. Mehta, Negligible colon cancer risk from food-borne acrylamide exposure in male F344 rats and nude (nu/nu) mice-bearing human colon tumor xenografts, PLoS One 8 (2013) e73916.
[22] J. Raju, C. Sondagar, J. Roberts, S.A. Aziz, D. Caldwell, E. Vavasour, R. Mehta, Dietary acrylamide does not increase colon aberrant crypt foci formation in male F344 rats, Food Chem. Toxicol. 49 (2011) 1373–1380.
[23] J. Raju, J. Roberts, M. Taylor, D. Patry, E. Chomyshyn, D. Caldwell, G. Cooke, R. Mehta, Toxicological effects of short-term dietary acrylamide exposure in male F344 rats, Environ. Toxicol. Pharmacol. 39 (2015) 85–92.
[24] V. Sharma, J.H. McNeill, To scale or not to scale: the principles of dose extrapolation, Br. J. Pharmacol. 157 (2009) 907–921.
[25] Q. Sheng, H.C. Zou, Z.R. Lü, F. Zou, Y.D. Park, Y.B. Yan, S.J. Yao, Effects of acrylamide on the activity and structure of human brain creatine kinase, Int. J. Mol. Sci. 10 (2009) 2422–4210.
[26] A. Shipp, G. Lawrence, R. Gentry, T. McDonald, H. Bartow, J. Bounds, N. Macdonald, H. Clewell, B. Allen, C. Van Landingham, Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects, Crit. Rev. Toxicol. 36 (2006) 481–608.
[27] F. Sörgel, R. Weissenbacher, M. Kinzig-Schippers, A. Hofmann, M. Illauer, A. Skott, C. Landersdorfer, Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans, Chemotherapy 48 (2002) 267–274.
[28] R.H. Stadler, I. Blank, N. Varga, F. Robert, J. Hau, P.A. Guy, M.C. Robert, S. Riediker, Acrylamide from Maillard reaction products, Nature 419 (2002) 449–450.
[29] Swedish National Food Agency, Swedish National Food Agency, Acrylamide in Food, Uppsala, Sweden, 2002.
[30] S. Takami, T. Imai, Y.M. Cho, K. Ogawa, M. Hirose, A. Nishikawa, Juvenile rats do not exhibit elevated sensitivity to acrylamide toxicity after oral administration for 12 weeks, J. Appl. Toxicol. 32 (12) (2012) 959–967.
[31] USFDA, Guidance for Industry: Estimating the Maximum Safe Starting Dose in Adult Healthy Volunteer, US Food and Drug Administration, Rockville, MD, 2005.
[32] USFDA, Survey Data on Acrylamide in Food: Total Diet Study Results, 2006 http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm053566.htm#table4.
[33] USFDA, The 2006 Exposure Assessment for Acrylamide, 2006 http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM197239.pdf.
[34] W.W. Wong, S.W. Chung, C.H. Lam, Y.Y. Ho, Y. Xiao, Dietary exposure of hong kong adults to acrylamide: results of the first Hong Kong total diet study, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 31 (5) (2015) 799–805.