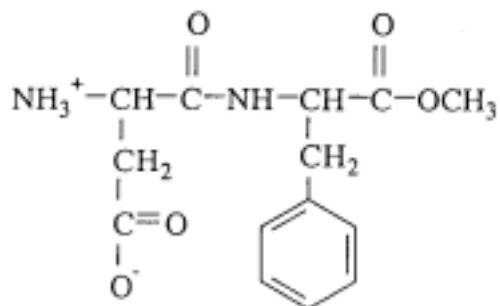


Carcinogenicity and Toxicity Evaluation of Aspartame in the heterozygous p53 deficient(+/-) mouse, hemizygous Tg. AC (+/-) mouse and the heterozygous p16 (+/-) mouse

Test chemical: Aspartame

CAS Number: 22839-47-0

Chemical Structure:



Molecular Formula: C₁₄-H₁₈-N₂-O₅

Molecular weight: 294.3

Experimental model systems:

- heterozygous p53 deficient (+/-) mouse (1-4)
- hemizygous Tg.AC mouse (5-7)
- heterozygous p 16 (+/-) mouse

Purpose:

This study will determine the effect of aspartame in the heterozygous p53 deficient (+/-) mouse, hemizygous Tg. AC (+/-) mouse and the heterozygous p 16 (+/-) mouse. These studies will be conducted according to GLP conditions and requirements. NTP 2000 feed shall be used for these studies.

a). p53 (+/-) mouse

After a ten to fourteen day quarantine period, animals shall be assigned at random to treatment and control groups. At each of five concentrations plus a control group, fifteen animals per sex shall receive aspartame in the feed for 39-weeks. Mice shall be housed individually.

Aspartame will be given in the feed at 3125, 6250, 12,500, 25,000, and 50,000 ppm

	<u>Animals</u>	<u>Sexes</u>	<u>Test Groups</u>	<u>Total</u>
Treatment	15 x	2 x	x 5	150
Controls	15 x	2 x	x 1	<u>30</u>
Total				180

b). Tg. AC mouse

After a ten to fourteen day quarantine period, animals shall be assigned at random to treatment and control groups. At each of five concentrations plus a control group, fifteen animals per sex per species shall receive aspartame in the feed for 39-weeks. Mice shall be housed individually.

Aspartame will be given in the feed at 3125, 6250, 12,500, 25,000 or 50,000 ppm.

	<u>Animals</u>	<u>Sexes</u>	<u>Test Groups</u>	<u>Total</u>
Treatment	15 x	2 x	x 5	150
Controls	15 x	2 x	x 1	30
TPA control*	15 x	2 x	x 1	<u>30</u>
Total				210

* TPA - positive control - 1.25 ug TPA mouse 3X/week (in a volume up to 100 ul acetone). TPA treated animals shall be terminated when they have > 20 papillomas.

c). P16 (+/-)mouse

After a ten to fourteen day quarantine period, animals shall be assigned at random to treatment and control groups. At each of five concentrations plus a control group, fifteen animals per sex shall receive aspartame in the feed for 39-weeks. Mice shall be housed individually.

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Aspartame will be given in the feed at 3125, 6250, 12,500, 25,000, and 50,000 ppm

	<u>Animals</u>	<u>Sexes</u>	<u>Test Groups</u>	<u>Total</u>
Treatment	15 x	2 x	x 5	150
Controls	15 x	2 x	x 1	<u>30</u>
 Total				 180

2. Observations:

Animals shall be weighed individually on day one on test, after seven days and at weekly periods thereafter. The animals shall be observed twice daily, once in the early morning and once in the late afternoon, at least six hours apart (before 10:00 AM and after 2:00 PM), including holidays and weekends for signs of morbidity and death. Signs of toxicity noticed during these routine checks shall be recorded. Formal clinical observations shall be performed and recorded weekly. Feed consumption shall be measured weekly.

3. Necropsy and Histopathologic Evaluation:

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A complete necropsy shall be performed on all treated and control animals that either die or are sacrificed, and all tissues as listed in Attachment II of the SOW shall be fixed in formalin fixation for 24-48 hours, followed by transfer to 70% EtOH and embedding of protocol required tissues within 48 hours (this procedure is generally less harsh on antigenicity for any possible immunostaining). Organ weights shall be determined for all animals surviving until the end of the study. Those organs to be weighed are: brain, liver, thymus, right kidney, right testicle, heart, and lungs. Organs shall be weighed to the nearest 10 mg except for testis and thymus which shall be weighed to the nearest 1.0 mg. Microscopic pathology evaluations shall be performed on the list of tissues designated below plus potential treatment-related gross lesions for all mice. Preserved tissues from all test groups will be shipped to NIEHS/NTP Archives, RTP, NC.

Tissue List for Histopathology

Tissue list for trimming, sectioning, and H&E staining (by standard NTP SOW procedures unless otherwise stated):

Liver	Stomach** (glandular and non-glandular)
Kidneys	
Lungs	Adrenal/pituitary/thyroid
Spleen	Ovary/uterus or testis/epididymis
Thymus	Skin (SOA if applicable)
Lymph nodes (mesenteric/mediastinal)	Mammary gland
Heart	Gross lesions (masses)
Brain*	

* Special brain sectioning:

An extended evaluation of the brain (5 sections per animal) is to be conducted. In order that the same sections are consistently obtained from each animal, the specific landmarks for trimming and instructions for embedding/sectioning are described in detail in the attachment. Although 6 sections may be obtained from the described procedure, only sections designated numbers 1-5 by the protocol are necessary.

Note: This procedure requires the use of a brain trimming matrix that the laboratory conducting the studies will need to obtain. It is recommended that practice trimming of brains from non-study mice be done prior to those from study necropsies.

** Special stomach sectioning:

Stomachs are to be opened along the greater curvature at necropsy, rinsed free of contents with saline or fixative, pinned flat onto a firm surface (e.g., heavy cardboard), and immersion fixed flat. Following fixation, a longitudinal strip through the center of the opened stomach and including both glandular and nonglandular compartments is to be obtained and embedded on edge for sectioning.

Attachment II of the NTP Statement of Work

Complete gross necropsy is defined as external examination of the animal including body orifices and examination and fixation of all of the following organs/tissues from animals from all treatment groups in all studies for histopathologic examination:

Adrenal glands	Nose (nasal cavity and nasal turbinates)
Brain	Oral cavity, larynx and pharynx
Clitoral glands	Ovaries
Esophagus	Pancreas
Eyes	Parathyroid glands
Femur	Pituitary gland
Gallbladder (mouse)	Preputial glands
Gross lesions	Prostate
Harderian gland	Salivary glands
Heart and aorta	Seminal vesicles
Intestine, large (cecum, colon, rectum)	Skin (skin paint studies only)
Intestine, small (duodenum, jejunum, ileum)	Spinal cord Spleen Stomach (forestomach and glandular)
Kidneys	Testes, epididymis and vaginal tunics of testes
Liver	Thymus
Lungs and mainstem bronchi	Thyroid glands
Lymph nodes	Tissue masses
- mandibular and mesenteric -bronchial and mediastinal (inhalation studies)	Tongue
- - inguinal, gluteal, internal iliac (chronic only, if obvious discoloration from tattoo)	Trachea
Mammary gland with adjacent skin	Urinary bladder
Muscle, thigh	Uterus
Nerve, sciatic	Vagina
	Zymbal glands

Samples of control and treatment-related tumors (0.5 - 1 cm diameter) , and corresponding "normal tissue" from the same organs, will be flash frozen in labeled cryovials in liquid nitrogen and saved in liquid nitrogen vapor for possible molecular biology studies. The ears of each animal from which a frozen specimen is taken shall also be frozen for use in confirming the genotype of the animal. An inventory of the tissues/tumors saved shall be provided to the project officer.

4. Erythrocyte Micronuclei Determinations

Two unstained blood smears shall be prepared from all mice at necropsy for use by the NTP in micronuclei determinations. The slides will be fixed in 100 percent methanol and sent to the NTP designated contractor.

5. Determination of plasma levels of chemical

Blood samples will be obtained at necropsy from all animals and plasma harvested and frozen for the determination of plasma levels of chemical /metabolite. Frozen plasma samples shall be shipped to analytical contractor designated by NIEHS.

Pre-labeled blood collection tubes (animal number, experiment number, and blood collection time) shall be available, and covered with paraffin (in order to eliminate contamination).

It is desirable to start necropsy as soon as possible in the morning (7 AM if possible) and equal number of animals from each dose group necropsied at 7 AM, 8 AM etc. Time of necropsy should be noted on plasma vial and on necropsy sheet.