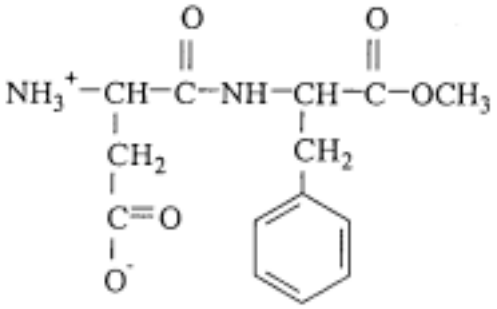
Carcinogenicity and Toxicity Evaluation of Aspartame in the heterozygous p53 deficient(+/-) mouse, hemizygous Tg. AC (+/-) mouse and the heterozygous p16 (+/-) mouse

Test chemical: Aspartame

CAS Number: 22839-47-0

Chemical Structure:



Molecular Formula: C_{14}-H_{18}-N_2-0_5

Molecular weight: 294.3

Experimental model systems:

a). heterozygous p53 deficient (+/-) mouse (1-4)
b). hemizygous Tg.AC mouse (5-7)
c). 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 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

	Animals	Sexes	Test Groups	Total
Treatment	15 x	2 x	x 5	150
Controls	15 x	2 x	x 1	30
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.

	Animals	Sexes	Test Groups	Total
Treatment	15 x	2 x	x 5	150
Controls	15 x	2 x	x 1	30
TPA control*	15 x	2 x	x 1	30
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.

[1]

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

	Animals	Sexes	Test Groups	Total
Treatment	15 x	2 x	x 5	150
Controls	15 x	2 x	x 1	30
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:

[2D]

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
Kidneys
Lungs
Spleen
Thymus
Lymph nodes (mesenteric/mediastinal
Heart
Brain*

Stomach** (glandular and non-glandular)

Adrenal/pituitary/thyroid
Ovary/uterus or testis/epididymis
Skin (SOA if applicable)
Mammary gland
Gross lesions (masses)

* 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:

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Adrenal glands
Brain
Clitoral glands
Esophagus
Eyes
Femur
Gallbladder (mouse)
Gross lesions
Harderian gland
Heart and aorta
Intestine, large (cecum, colon, rectum)
Intestine, small (duodenum, jejunum, ileum)
Kidneys
Liver
Lungs and mainstem bronchi
Lymph nodes
- mandibular and mesenteric -bronchial and mediastinal (inhalation studies)
- - inguinal, gluteal, internal iliac (chronic only, if obvious discoloration from tattoo)

Mammary gland with adjacent skin Muscle, thigh Nerve, sciatic
Nose (nasal cavity and nasal turbinates
Oral cavity, larynx and pharynx
Ovaries
Pancreas
Parathyrold glands
Pituitary gland
Preputial glands
Prostate
Salivary glands
Seminal vesicles
Skin (skin paint studies only)
Spinal cord Spleen Stomach (forestomach and glandular)
Testes, epididymis and vaginal tunics of testes
Thymus
Thyroid glands
Tissue masses
Tongue
Trachea
Urinary bladder
Uterus
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.