PHENYLACETIC ACID

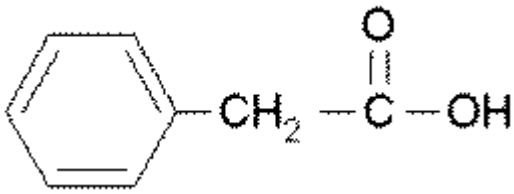
SYNONYMS

Acetic acid, phenyl-
Alpha-toluic acid
Benzeneacetic acid
Benzylcarboxylic acid
Ω-phenylacetic acid
Phenylacetic acid
Phenylethanoic acid

CHEMICAL FORMULA

$C_8H_8O_2$

CHEMICAL STRUCTURE



IDENTIFIER DETAILS

CAS Number	:	103-82-2
CoE Number	:	672
FEMA	:	2878
EINECS Number	:	203-148-6
E Number	:	-

SPECIFICATIONS

Melting Point:	76 -78 C	[Sigma-Aldrich, 2002]
Boiling point:	265 C	[Sigma-Aldrich, 2002]
Smiles code:	c1(ccccc1)CC(O)=O	
Log P:	1.41	[ToxNet, 2011]

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
5	30	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Acceptable	JECFA	2002	No safety concern at current levels of intake when used as a Flavouring agent

FDA Status:[CFR21]

Section Number	Comments
172.515	Synthetic flavouring substances and adjuvants

HUMAN EXPOSURE

Natural Occurrence: Phenylacetic acid is reportedly found among the constituents of a few essential oils: tobacco, *Rosa centifolia*, Bulgarian rose, orange flowers absolute, neroli, and *Mentha arvensis* of Japanese origin; also reported present among the volatile constituents of cocoa. Phenyacetic acid is also reportedly found in a wide range of foods including guava, raspberry, mango and mushroom [Fenaroli, 2005].

Reported Uses: Phenylacetic acid is reported ly used in baked goods at 12.32 ppm, frozen dairy at 8.61 ppm, meat products at 1.3 ppm, soft candy at 13.88 ppm, sweet sauce at 20 ppm, gelatin, pudding at 5.1 ppm, non-alcoholic beverages at 2.12 ppm, alcoholic beverages 4.76 ppm, and hard candy at 3.17 ppm [Fenaroli, 2005].

TOXICITY DATA

The GRAS status of Phenethyl alcohol has been recently reassessed by the FEMA Expert Panel [Adams, 2005]. The panel stated that "The group of phenethylalcohol, aldehyde, acid, and related acetals and esters was reaffirmed as GRAS (GRASr) based, in part, on their self-limiting properties as flavoring substances in food; their rapid absorption, metabolic detoxication, and excretion in humans and other animals; their low level of flavour use; the wide margins of safety between the conservative estimates of intake and the no-observed-adverse effect levels determined from subchronic and chronic studies and the lack of significant genotoxic and mutagenic potential" [Adams, 2005]. Furthermore, the panel add that "This evidence of safety is supported by the fact that the intake of phenethyl alcohol, aldehyde, acid, and related acetals and esters as natural components of traditional foods is greater than their intake as intentionally added flavouring substances" [Adams, 2005].

Carmines (2002), Rustemeier *et al*., (2002), Roemer *et al*., (2002) and Vanscheeuwijck *et al.,* (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the

combined ingredients, including Phenylacetic acid at levels up to 96 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 2002].

***In Vivo* Toxicity Status**

Species	Test Type	Route	Reported Dosage
Rat	LD_{50}	Intraperitoneal	1600mg/kg
Mouse	LD_{50}	Intraperitoneal	2250mg/kg
Mouse	LD_{50}	Intraperitoneal	2270mg/kg
Mouse	LD_{50}	Subcutaneous	1500mg/kg
Guinea Pig [Lewis, 2000]	LD_{50}	Subcutaneous	2250mg/kg
Rat	TD_{LO}	Oral	450mg/kg *

* (4 day administration. During pregnancy teratogenic effects e.g. stunted foetus) (RTECS, 2002).

The single administration of 375-750 mg/kg/bw phenylacetic acid to guinea-pig was observed to decrease blood sugar levels, with no other side-effects, [BIBRA, 1989]. Mice given phenylacetic acid at 100-300 mg/kg/bw [intraperitoneal] were reported to induce respiratory failure and death, [BIBRA, 1989].

Animal studies in which s.c. injections of 200-340 mg /kg/bw phenylacetic acid administered to rats from 2-21 days , was observed to alter bio chemical processes in the brain [BIBRA, 1989].

Rats [unspecified number] given 500-1000 mg 'phenylacetate' kg/bw/day for a period of 13-14 days were reported to have 'liver changes ' in the 500 mg/kg/bw group, [the paper did not state if phenylacetic acid was administered as the salt or the phenyl ester of acetic acid]. Similarly rats given 1500-2500 mg/kg/bw phenyl acetic acid a day for 1-3 weeks were only reported to have minor biochemical alterations [changes in the serotonin levels] [BIBRA, 1989].

The i.p. injection of phenylacetic acid in 6 rats over a 7 day period [150 mg/kg/bw/ day] did not induce any liver changes [this was the only organ examined at the termination of the study], [BIBRA, 1989]. Juvenile rats [whose detoxification path way for phenylacetic acid was not yet developed] when given 200-340 mg/kg/bw/dy for short periods [time unspecified] , were reported to be lethargic and irritable [this was reported to be accompanied with a decreased brain weight] and alterations in brain a nd eye development were noted, [no other organs were examined] [BIBRA, 1989].

Human data (oral exposure):

Dosage	Effect observed
~71 mg/kg/bw	Immediate thirst , dizziness and nervousness [if no food eaten]. Nausea observed if food was eaten
~50 mg/kg/bw	No effects observed [single case]
~166 mg/kg/bw	No effects observed [single case]
~230 mg/kg/bw	No effect observed [single case]
~230 mg/kg/bw	Headache, eye pain, ringing ears, inability to stand upright [one case].

In all of the above cases th e number of volunteers per group was unknown unless stated, [BIBRA, 1989].

Carcinogenicity and Mutagenicity

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combinatio n, including phenylacetic acid at 22 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate " [It should be noted that the cigarettes contained a typical A merican blend humectant and sugar component (*i.e.* glycerine 20,000 ppm, propylene glycol at 24,000 ppm, and brown invert sugar at 24,000 ppm)] [Gaworski *et al.*, 1999].

Phenylacetic acid [unspecified dose] on administration to rabbits [intravenous or subcutaneous 40 day study] did not show any signs of tumour promotion, [HSDB, 2002].

Dermal Toxicity

Studies involving the application of 2 % phenyl acetic acid in petroleum to 25 volunteers [5x 48h patch test for a period of 10 days] and then re-applic ation after a rest period of 10-14 days [48-hr covered patch] produced no local reactions, therefore giving no evidence of sensitisation, [BIBRA, 1989]. A similar study with an undisclosed number of volunteers [48 hr covered patch], documented a slight irritant effect [however no other data was given], [BIBRA, 1989].

A study in which 100 mg phenylacetic acid was placed into the rabbit eye [number and strain not given] revealed it to be a moderate irritant, [HSDB, 2002].

Reproductive and Developmental Toxicity

Rat receiving 450 mg/kg/bw/dy phenylacetic acid throughout pregnancy were observed to produce foetotoxicity as indicated by retarded ossification, decreased foetal weight and increased early foetal deaths. Delayed ossification was also seen at 45 mg/ kg bw/dy but not at 4.5 mg/kg bw/dy, [BIBRA, 1989]. A later post-implantation study in which 10 day whole rat embryos [4 or 8 somites] were cultured in rat serum and the effects of added

phenyl acetic acid was observed [after a 26 h culture, with test comp ound] indicated that phenylacetic acid induced dose-related embrotoxicity at concentrations above 0.3 mg/ml, [HSDB, 2002].

A teratogenicity study in which phenylacetic acid was administered to rats on the 12 th day of embryogenesis revealed an affected bod y weight, retarded ossification, and a resorption rate twice that of control animals. The dosage level was 0.2 % of LD_{50} or 3.2 mg/kg, [HSDB, 2002]. It has also been reported that phenylacetic acid has a LC_{50} of 1,273.7 mg/ml [95 % confidence intervals of 1,252-1,296], EC_{50} [for malformations] of 801.9 mg/ml [95 % confidence intervals of 761-845] and a DHI [developmental hazard index] at 96 h of 1.6 as reported from a developmental study using *Xenopus* embryos exposed to acid solution for 96 hours], [Dawson *et al.,* 1996]. Similarly phenylacetic acid was observed to have a developmental hazard index of 2 as determined in the hydra assay [Note: Compounds with index ratios 3 are further tested in a mammalian system], [Johnson *et al*., 1988].

Four groups of 10 rats were orally administered phenyl acetic Acid at 0, 250, 500 and 1000 mg/kg/day 7 days prior to cohabitation, through cohabitation (maximum 7 days), gestation, delivery and 4 days postpartuition. Those rats that did not deliver a litter were necropsied on Day 25, with delivered pups being sacrificed on day 4 postpartum. The NOAEL for maternal toxicity was <250 mg/kg/day and developmental offspring effects was 500 mg/kg/day [Vollmuth *et al.,* 1990].

Neuralating mouse embryos were exposed to phenylalanine (Phe) and Phe metabolites (phenylethylamine, phenylpyruvic acid and phenylacetic acid), to assess teratogenicity at concentrations ranging from 0.01mM to 10mM for 24 hours. Embryos were examined for morphological abnormalities and protein content by the Lo wry method. No Neural Tube closure defects (NTDs) were observed for phenylacetic acid at concentrations of 1mM, 60% at 5mM, and 100% at 10mM. Phenylalanine and Phe metabolites produced a significant reduction in embryonic protein, [Denno & Sadler., 1990].

Inhalation Toxicity

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90-day nose-only smoke inhalation study in rats. These ingredients included phenylacetic acid at 96 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study, along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the s moke, even at the exaggerated levels used " [Vanscheeuwijck *et al.*, 2002].

When tested at 22 ppm in cigarettes, in a 13-week inhalation study, the presence of phenylacetic acid "…had no discernible effect on the character of extent of the biologic response s normally associated with inhalation of mainstream cigarette smoke in rats. "[Gaworski *et al.* , 1998] [However, it should be noted that the cigarettes had been spiked with a number of flavour

ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component (*i.e.* glycerine 20,000 ppm, propylene glycol at 24,000 ppm, and brown invert sugar at 24,000 ppm)] "[Gaworski *et al.*, 1998].

The addition of phenylacetic acid at 76 ppm to reference cigarettes , used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of phenylacetic acid to a re ference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

Other Relevant Studies

Phenylacetic acid, a metabolite of 2-phenyl ethylamin e, acts as a neuromodulator in the nigrostriatal dopaminergic pathway stimulating the release of dopamine. The evaluation of phenyl acetic acid concentration in the biological fluid reflects phenyl ethylamine levels thus allowing the assessment of the modu latory role of this endogenous substance. Changes in biological fluids levels of 2-phenylethylamine and/or in its metabolite have been reported in affective disorders, such as depression and schizophrenia. Recently, the occurrence of the "attention deficit hyperactivity syndrome" has been frequently reported in childhood population and involvement of dopaminergic dysfunction in this disease has been suspected [Mangani *et al.,* 2004].

The dose limiting toxicity and pharmacokinetics of phenylacetic acid [administered as phenylacetate, dose not stated] was studied in 17 patients, all of which had solid tumours and received single intravenous bolus doses followed by continuous infusion of the acid over 14 days. Although 99 % of the phenylacetic acid was excreted in the urine, continuous intravenous infusion resulted in drug accumulation and dose limiting toxicity consisting of reversible CNS depression usually preceded by emesis. Stable maintenance of phenylacetic acid concentrations of up to 200-300 g/ml was reported to result in the clinical improvement of advanced disease, [HSDB No: 5010].

Neish *et al* ., (1971) suggested that phenyl acetic acid could be a potential therapeutic agent in the treatment of cancer as it is able to deplete glutamine reserves of cancer patients, [Neish *et al*., 1971].

Volunteers [unspecified sample size] given 1mg/kg bw phenylacetic acid excreted 90-92 % in the urine as the glutamine conjugate, with the remainder conjugated with taurine or glycine, [BIBRA, 1989]. Phenylacetic acid is also reported to be rapidly absorbed from human buccal tissues membranes, [HSDB, 2002].

Phenylacetic acid has been shown to build up to abnormally high levels in patients with phenylketonuria. This is implicated as a major cause of mental retardation in phenylketonuria patients, [BIBRA, 1989].

Phenylacetic acid was present in 47.3 % of the 54 samples of normal human expired air from an average concentration of 0.161ng/L, [no other details given, [HSDB No: 5010].

Nitric oxide [NO] prevents atherogenesis and inflammation in vessel walls by inhibition of cell proliferation and cytokine-induced endothelial expression of adhesion molecules and proinflammatory cytokines. Reduced NO production due to inhibition of either eNOS or iNOS may therefore reinforce atherosclerosis. Patients with end-stage renal failure show markedly increased mortality due to atherosclerosis. In the present study we tested the hypothesis that uremic toxins are responsible for reduced iNOS expression. LPS-induced iNOS expression in mononuclear leukocytes was studied using real-time PCR. The iNOS expression was blocked by addition of plasma from patients with end-stage renal failure, whereas plasma from healthy controls had no effect. Hemofiltrate obtained from patients with end-stage ren al failure was fractionated by chromatographic methods. The chromatographic procedures revealed a homogenous fraction that inhibits iNOS expression. Using gas chromatography/mass spectrometry, this inhibitor was identified as phenylacetic acid. Authentic p henylacetic acid inhibited iNOS expression in a dose-dependent manner. In healthy control subjects, plasma concentrations were below the detection level, whereas patients with end-stage renal failure had a phenylacetic acid concentration of 3.49 +/- 0.33 m mol/l (n = 41). It is concluded that accumulation of phenylacetic acid in patients with end-stage renal failure inhibits iNOS expression. That mechanism may contribute to increased atherosclerosis and cardiovascular morbidity in patients with end-stage renal failure [Jankowski *et al*., 2003].

A rapid screening protocol was used to evaluate the potential immunotoxicity of flavouring ingredients, including phenylacetic acid. The protocol incorporated key elements of the National Toxicology Program 's tier test ing strategy, including measurement of body weight, lymphoid organ weight and cellularity, as well as functional tests of the humoral (antibody plaque-forming cells) response to sheep erythrocytes and cell-mediated immunity to *Listeria monocytogenes* bacterial challenge. Decreases in body weight, spleen and/or thymus weight or a decrease in spleen cellularity may be indicative of depressed immune competence. The number of antibody-producing plasma cells, the result of antigen-driven B-cell differentiation, a fter immunization with a T-cell-dependent antigen such as sheep red blood cells, provides information about the functional integrity of, and communication among, several cell populations important in antibody-mediated immunity, including T cells, B cells a nd macrophages. The *Listeria* model system was selected because the pathogenesis of this infection and the host 's immune response are similar in mice and humans. The model system is useful for assessing immunosuppression since both immunocompetent T cells a nd macrophages are required to control infection and supply protective immunity. Phenylacetic acid administered orally to groups of 10 –20 female CD-1 or B6C3F $_1$ mice, aged 6 –8 weeks, at doses as high as 100 mg/kg per day had no effect on spleen weight, thym us weight, spleen cellularity, anti-sheep red blood cell response or *Listeria* mortality [Vollmuth et al., 1989].

Phenylacetic acid was shown to be devoid of immunomodulatory effects in a testing strategy to evaluate the effects of 35 commonly used flavouri ng ingredients on humoral and cell-mediated immune responses. Female CD-1 or $B6C3F_1$ mice were given phenylacetic acid at a dose of 250, 500 or 1000 mg/kg bw per day intragastrically for 5 days. *L. monocytogenes* bacterial challenge was conducted to assess c ell-mediated immunity and the antibody response to sheep erythrocytes was determined as a measure of humoral immunity. Body weights, lymphoid organ weights and spleen cellularity were also measured. Cyclophosphamide served as a positive control. Phenylacet ic acid did not modulate the cell-mediated or humor al response at any dose tested [Gaworski et al., 1994].

In a study of the effects of excess L-phenylalanine, L-tyrosine, L-valine and phenylacetic acid on serotonin in brain and liver, 5% or 7% phenylaceti c acid in the diet for 1 –3 weeks increased brain serotonin and dec reased liver serotonin in rats [Boggs et al., 1963].

Behavioural data

No data identified

In Vitro **Toxicity Status**

Carcinogenicity and Mutagenicity

Microarray anal. revealed that treatme nt of P. aeruginosa with PAA down-regulated the transcriptional expression of type III secretion system (T3SS) genes and related regulatory genes including rsmA and vfr, which were confirmed by transcriptional and translational anal. Identification of bact erial metabolite PAA as a T3SS-specific inhibitor explains this intriguing inverse cell-d.-dependent-cytotoxicity phenomenon as T3SS is known to be a key virulence factor assocd. with cytotoxicity and acute infection. The findings may provide useful clues for design and development of new strategies to combat this formidable bacterial pathogen. [on SciFinder(R)]

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the Salmonella plate incorporation (Ames) assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitr o* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included pheny lacetic acid at levels up to 96 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002].

Baker *et al.,* [200 4]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke.

The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of phenyl acetic acid at 76 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with th at of the control cigarettes [Baker *et al.,* 2004].
Heck and colleagues tested the mutagenic capabilities of phenylacetic acid in three different test systems; reverse mutation assay, unscheduled DNA synthesis and the mouse lymphoma assay. Phenylacetic acid was tested for its ability to induce reverse mutation in various strains of *Salmonella typhimurium* (e.g., TA98, TA100, TA1535, TA1537 and TA1538) in the presence or absence of an exogenous metabolic activation system. Heck et al., 1989; no increase in un scheduled DNA synthesis was noted when rat hepatocytes were incubated with phenylacetic acid (Heck et al., 1989).

Additional information concerning the *in vitro* mutagenicity of this material may be found in "An Interim report on data originating from Impe rial Tobacco Limited's Genotoxicity testing programme September 2003 " or "An updated report on data originating from Imperial Tobacco Limited 's external Genotoxicity testing programme – Round 2 August 2007".

The mutagenicity of the smoke condensate was as sayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included *phenylacetic acid* at levels up to 9 ppm.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-21).

Other Relevant Studies

(-)-Epigallocatechin gallate (EGCg), a major component of green tea polyphenols inhibited the pro duction of phenylacetic acid at 0.5mg/mL in general anaerobic medium broth. In resting cells of *P.gingivalis* which produced phenyl acetic acid from 1-phenylalanine and phenylpyruvic acid, phenyl acetic acid was also inhibited by EGCg. However, this did not occur in the presence of (+)-catechin, (+)-gallocatechin, (-)-epicatechin and (-)-epigallocatechin. The author concluded that the presence of the galloyl moiety ester linked with the 3-OH of the catechin moiety was responsible for the inhibitory effects observed [Sakanaka & Okada, 2004].
Phenylacetic acids such as 3,4-dihydroxy-phenylacetic acid showed a time and dose dependent inhibitory effect on cell growth in T47D human breast cancer cell lines. Inhibition of nitric oxide synthase and induction of apoptosis via the Fas/FasL system exerted a direct antiproliferative effect, at low concentrations comparable to phenolic acids found in biological fluids after ingestion of foods containing phenolic acids [Kampa *et al.,* 2004].

Phenylbutyrate (PB) and phenylacetate (PA) have antiproliferative and differentiation-inducing effects in malignant tumors, and had been evaluated in Phase I/II clinical trials. Li *et al,.* (2004) evaluated their antitum or activities in medulloblastomas. The biological effects of PB and PA, ranging from 0.1 mM to 3 mM, on two medulloblastoma cell lines (DAOY and D283-MED) were examined using various long-term *in vitro* and *in vivo* assays for morphology, proliferation, diff erentiation, anchorage-independent growth, apoptosis, and tumorigenicity. PB and PA could both induce morphological changes and suppress proliferation in a time- and dose-dependent manner. These effects were more pronounced with PB and became irreversible in D283-MED cells after continuous exposure to 3 mM PB for 28 days. Both PB and PA were able to increase expression of glial marker glial fibriliary acidic protein and neuronal marker synaptophysin in two cell lines. For anchorage-independent growth, PB sh owed a more significant suppression than PA in D283-MED cells. PB caused more pronounced cell cycle arrest and remarkably reduced tumorigenicity in D283-MED cells than in DAOY cells. Apoptosis was readily induced in D283-MED cells with either low dose of PB or short-term treatment. In contrast, much higher concentrations of PB or longer treatment were required to achieve similar effect with DAOY cells. PB induced increased histones H3 acetylation in both cell lines, but histone H4 acetylation was only observed in D283-MED cells. PB, through induction of hyperacetylation of histone H3 and H4, was a much more potent antitumor agent than PA. 283-MED cells were more responsive to PB than DAOY cells, which may be dependent on their original state of differentiati on as well as the changes of histone H4 acetylation status [Li *et al.,* 2004].

The effect of phenylacetic acid (PAA) (a uraemic toxin) on inducible nitric oxide synthase (iNOS) and the production of reactive oxygen species (ROS) was investigated in end sta ge renal disease patients (ESRD). Vascular smooth muscle cells (VSMC) were stimulated by IL-1 in the absence / presence of PAA (0.1-5.0mM). iNOS mRNA was determined using RT-PCR, iNOS protein using western blotting and NO degradation products (nitrite), peroxynitrite, the iNOS cofactor tetrahydrobiopterin (BH(4)) and the expression of the main enzyme GTP cyclohydrolase (GTPCH). Treatment with PAA resulted in an increase in IL-1 mediated induction of iNOS expression (mRNA and protein). Nitrite was also sig nificantly increased at the highest concentration of PAA (5mM) and peroxynitrite was enhanced in a dose dependent manner. PAA caused no increase in GTPCH associated with BH(4) synthesis. In conclusion oxidative stress in ESRD may result from PAA increased iNOS expression and peroxynitrite production possibly due to the lack of GTPCH catalysed BH (4) syntheses [Schmidt *el al.,* 2008].

Phenylacetic acid (PAA) has been identified as one of the uraemic toxins in patients with chronic kidney disease and has an inhibiting property of monocyte function. The authors examined if PAA might inhibit osteoblast ic functions *in vitro* using mouse osteoblastic MC3T3-E1 cells, PAA significantly inhibited proliferation in a dose-dependent manner ranging between 0.5 and 5 mM. PAA reduced osteocalcin mRNA level, alkaline phosphatase activity and osteoblastic mineralization. PAA pre-treatment also decreased both

Parathyroid hormone (PTH)-induced cAMP production and extracellular signal-regulated kinase (ERK) phosphorylation. The authors conclude a newly identified uraemic toxin, affects osteoblastic functions such as proliferation, differentiation, mineralization and responsiveness to PTH, indicating that this molecule could play an important role in the pathogenesis of low turnover bone in CKD (Tano *et al*., 2007)

Schmidt *et al*., (2008) investigated the impact of penylacetic acid (PAA) on macrophage function. The phagocytic activity of RAW 264.7 cells was not significantly affected by PAA, whereas the cytotoxicity against intracellular bacteria was significantly reduced. The author's findings show that the uraemic toxin PAA has inhibitory effects on macrophage-killing function, which are mediated by inhibitory effects on transcriptional iNitrous Oxide Synthase regulation. iNOS inhibition by PAA might affect immunoregulatory processes and could play a role in aggravation of immunodeficiency of patients with end-stage renal disease (Schmidt *et al*., 2008).

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of phenylacetic acid is detailed in the Report on Thermochemical Properties of Ingredients document. In the aforementioned document, the term 'pyrolysis' means the heating of an ingredient in isolation under controlled conditions in an analytical device to examine its degradation potential. The expression 'transfer data' on the other hand is used to describe the fate of an ingredient in qualitative and quantitative terms following the smoking of a tobacco product to which it has been applied.

REACH Statement

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

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