

While stability may be important for sweet protein functionality, stability may also correlate with potential food allergenicity or toxicity. To assess potential allergenicity, a structural homologue of our AFPs was purified from wheat (gamma-thionin) and directly tested for allergenicity using IgE from wheat-allergic patients. In addition, acute oral toxicity studies of sweet proteins in mice demonstrated a lack of toxic effect at high doses. Data from these experiments are being used to direct new inquiries into the safety and function of plant AFPs and sweet proteins.

1951 ABSORPTION OF CADMIUM FROM INFANT DIETS IN NEWBORN RATS.

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Infants that are not breast-fed have a higher exposure of cadmium (Cd) than infants given breast milk from infant- and follow-on formulas. The bioavailability of Cd from such formulas was studied in rat pups. Cow's milk formula, soy formula, wheat/oat/milk formula, wholemeal/milk formula and water were labelled with ¹¹⁰⁹Cd. Eleven days old rats received a single oral dose of one diet containing 3µCi (0.1mg Cd/kg bw) or 10µCi (0.3mg Cd/kg bw). After 2 or 24 hours or 4, 9 or 12 days the retention of ¹¹⁰⁹Cd in the whole body, in segments of the rinsed small intestines and in tissues was measured in a gamma counter. Pups receiving ¹¹⁰⁹Cd in water or cow's milk formula had the highest whole body retention at all survival times. The retention 4 days after dosage ranged from approximately 70% of the dose in the water group to 55% in the cereal-based formula groups. The retention in the rinsed small intestines, including the duodenum, jejunum and ileum, was very high still at 4 days (71-54%) and 9 days (24-11%). Initially most of the ¹¹⁰⁹Cd was retained in the duodenum but later it had moved further down in the jejunum. Four days after dosage the highest retention in most segments was found in the water- and cows' milk formula groups. There was an increase in ¹¹⁰⁹Cd in all tissues up to 4 days after administration and in the kidney still at 12 days. The uptake in the liver after 4 days in the water- and wholemeal/milk formula groups was 16 and 3% of the dose, respectively. The composition of the formula affected the bioavailability of ¹¹⁰⁹Cd so that it was higher from the cow's milk formula than from the cereal-based formulas. The lower uptake of Cd from cereal-based formulas might be explained by a high content of dietary fibres and phytic acid, binding Cd and reducing intestinal binding. The high retention of ¹¹⁰⁹Cd in the small intestines, leading to a prolonged absorption period stresses the importance of extending studies on neonatal Cd absorption over a long time period in order to detect e.g., the accumulation of Cd in the kidney.

1952 HEALTH RISKS TO RECREATIONAL HUNTERS CONSUMING RADIOCESIUM CONTAMINATED DEER AND HOGS FROM THE US DEPARTMENT OF ENERGY'S SAVANNAH RIVER SITE.

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Recreational hunters consuming radiocesium contaminated White-tailed deer (*Odocoileus virginianus*) and Feral swine (*Sus scrofa*) from the Department of Energy's (DOE's) Savannah River Site (SRS), can result in the transfer of the contaminant to humans. Radiocesium (Cs-137) is widespread across the SRS as a result of global atmospheric fallout from nuclear weapons testing and the former production of nuclear materials at the site. Because of the omnivorous feeding habits of the hogs, they can also acquire uptake from rooting and soil ingestion in radiocesium contaminated wetlands. Past studies of radiocesium have shown elevated levels in the bodies of game animals from the SRS. Deer and hogs are common inhabitants at the SRS and are regularly taken during the fall hunting season. Prior to release to the hunter, the deer and hogs are monitored to estimate Cs-137 levels in the game animal. The potential risk of contaminant intake from consuming deer and hogs from 1997 through 1999 was examined using various risk scenarios and mean meat and whole body concentrations of Cs-137. A human health risk analysis was performed based on the reasonable maximal exposure (RME) to potential hunters and their families from the consumption of the contaminated game meat. The annual committed effective dose equivalent (CEDE) associated with the ingestion of contaminated game meat by the hunter were as follows: deer = 8.5 mrem/year (85 µSv/year), and hog = 11.6 mrem/year (116 µSv/year), was far below the International Commission on Radiological Protection guideline of 100 mrem/year (1000 µSv/year). The cancer risk from ingestion of each contaminated game animal was 2E-04 for the recreational hunter. These analyses demonstrated the importance of properly identifying the particular target population at risk and their consumption patterns of meat from such wild game.

1953 VIRULENCE OF ISOLATES OF *LISTERIA MONOCYTOGENES* IN MURINE HEPATOCYTES.

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Listeria monocytogenes, a foodborne pathogen, causes listeriosis in humans and animals. Liver is a major target organ for the bacteria, where they invade and multiply within hepatocytes. There is a need for a better understanding of how *in vitro* assays relate to *in vivo* virulence. Therefore, the virulence of 3 food isolates (G3982, G3990 and H7550), 2 primate clinical isolates (12375 and 12443) and a human clinical isolate (Scott A) of *Listeria monocytogenes* was assessed in murine TIB73 hepatocytes. The virulent hemolysin positive strain (*Listeria hly+*) and avirulent hemolysin negative strain (*Listeria hly-*) were used as controls. Three of the isolates (12375, 12443 and G3982) exhibited greater virulence, two (G3990 and Scott A) similar virulence and one (H7550) less virulence in hepatocytes than *Listeria hly+*. The isolate 12443, which is the most virulent *in vivo*, has been found to induce stillbirth in experimentally infected rhesus macaques.

1954 *IN VITRO* SCREENING AND CHARACTERIZATION OF POTENTIAL SORBENTS FOR ZEAREALENONE.

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Previous methods for the control of zearalenone (ZEN) in animal feeds have proven largely ineffective in the treatment of ZEN-induced hyperestrogenism in animals. In this study, our objectives were threefold: 1) to screen structurally diverse clays (natural and chemically-modified) and other inorganic materials for their ability to remove ZEN from aqueous solution, 2) to identify and prioritize the best sorbents for ZEN, and 3) to assess and confirm the efficacy of sorbent-ZEN interactions using an adult hydra bioassay. In initial screening studies in water (pH 6.5 and 25°C), low levels of sorbents (0.002%) were tested for their potential to bind ZEN (4 ppm). Complete equilibrium adsorption isotherms were run for selected binding agents. The data were fitted to a variety of Langmuir-derived equations for the prediction of affinities and capacities of the binding agents. Our results indicated that the binding of ZEN onto the surfaces of various sorbents ranged from non-detectable to 99%. Among the 41 sorbents tested, activated carbon (less than 45 µ) showed the greatest amount of binding of ZEN (*i.e.*, 99%) followed by hectorite (a trioctahedral smectite), which showed approximately 22-28% binding. None of the other test sorbents demonstrated significant interaction with ZEN. The shape of the isotherm for activated carbon suggested that it predominantly sorbs ZEN by a partition process; whereas, hectorite exhibited an S-shape containing more than one plateau (and the potential for multiple sites and mechanisms). Q_{max} and K_d were estimated for the hectorite isotherm curve using a Langmuir model ($Q_{max} = 0.284$; $K_d = 4.75 \times 10^4$; $r^2 = 0.99$). The results of the adult hydra bioassay were in good agreement with the binding studies and confirmed the ability of carbon and hectorite for detoxification of ZEN. Based on these findings, hectorite should be further tested as a potential enterosorbent in animals for ZEN (Supported by USDA Grant 9703230 and TAES Project H-6215).

1955 AN ALTERNATIVE APPROACH FOR TOXICITY TESTING OF GENETICALLY MODIFIED FOODS.

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Food safety concerns are growing in the U.S. and abroad, due in part to the introduction of genetically modified (GM) foods into mainstream markets. Improved toxicity testing of GM food may help alleviate some of these concerns. Current toxicity testing for GM food, which is conducted and submitted to the FDA on a voluntary basis, consists of standard toxicological tests of the recombinant protein produced *in vitro*. Two primary criticisms of this approach are that 1) unintended genetic changes may occur in the whole plant, and, 2) the recombinant protein may be further modified by down-stream events occurring in the host plant. While the latter concern can be addressed by simply comparing the protein developed *in vitro* with the protein present *in vivo*, the former concern may require the development of an alternative toxicity testing protocol. We suggest a battery of whole food toxicity tests to fully address these concerns. Under this approach, GM food products and comparable non-GM food products (controls) grown under normal field conditions would be freeze dried and fed to laboratory animals for 30 days at doses ranging from 0 mg/kg to concentrations slightly less than those known to be toxic to animals due to the presence of natural compounds (*e.g.*, solanine). A battery of toxicological assays would be performed on the test and control groups, during and at study termination, including standard lethality, toxicity, pathophysiology, clinical chemistry, immune and enzyme induction assays. If significant differences be-

tween the groups were observed, then more rigorous testing would be proposed. If no significant differences were observed, however, this would suggest that public concerns over "unintentional" consequences associated with GM foods might not be justifiable. Another advantage of the proposed approach is that since it is based on "real-world" testing of GM foods, it allows for the evaluation of possible synergistic effects due to the co-occurrence of GM proteins in, and pesticides on, food.

1956 13 WEEK FEEDING STUDY IN RATS FED GRAIN FROM ROUNDUP READY CORN®

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This study was done to compare the responses of rats fed diets containing grain from Roundup Ready (RR) corn to rats fed (1) diets with grain from the parental variety (P) (non-transgenic) corn and (2) a series of diets with grain from commercial varieties (non-transgenic) corn, designated as reference controls, (RC). Male and female Sprague-Dawley rats (7 weeks of age, 20 rats/sex/group) were fed one of the following diets for 13 weeks: 11 or 33 % (wt/wt) RR ground corn grain; 11 or 33% P ground corn grain or 33% RC ground corn grain (6 commercial varieties were tested). There were a total of 10 groups or 400 rats in the study. All diets were formulated by Purina TestDiets (Richmond, Indiana) to be as similar as possible to Purina 5002 certified diets which contain approximately 33% ground corn grain. The diets which contained 11% RR or P grain were supplemented with 22% grain (non-transgenic commercial) corn to bring the total corn grain up to 33%, consistent with other diets. Grain samples and diets were analyzed for nutrient composition, pesticide residues and mycotoxins. All diets were balanced to be nutritionally equivalent. During the study, all animals were observed daily, body weight and food consumption were recorded at least weekly. After 4 and 13 weeks, blood and urine was collected from 10/sex/group for blood chemistry, hematology and urine analyses. After 13 weeks, all animals were sacrificed, necropsied and selected tissues weighed. Body weights and food consumption were comparable for all groups. There were no differences in organ weight or gross pathology findings. Clinical parameters (chemistry, hematology, urinalyses) were similar across groups with only a few exceptions. The few differences in clinical parameters were not considered biologically meaningful as they were either not dose related, or within the range of the reference controls. In conclusion, rats fed grain from RR corn responded similarly to rats fed P and RC grain.

1957 SAFETY ASSESSMENT OF MEPSPS PROTEIN IN ROUNDUP READY® CORN PLANTS.

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Glyphosate-tolerant corn (also known as Roundup Ready® corn) has been developed by Monsanto Company to enable the farmer to utilize Roundup® herbicide for effective control of weeds during the growing season and to take advantage of this herbicide's environmental and safety characteristics. Roundup Ready® corn line GA21 was produced by transformation with a gene encoding a glyphosate-tolerant, modified corn 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) protein gene fused to an optimized chloroplast transit peptide. The mEPSPS protein is 99.3% identical in its amino acid sequence to the wild-type maize enzyme and, except for its tolerance to glyphosate (higher K_i), is physically and functionally similar to maize and other plant EPSPs. EPSPS enzyme is ubiquitous in plants and has therefore been widely consumed safely. The safety of the mEPSPS protein was confirmed by evaluating physical, functional and toxicological characteristics. mEPSPS was digested rapidly *in vitro*, suggesting that it will be degraded in the mammalian digestive tract as a component of food or feed. Bioinformatics analyses showed that mEPSPS is not homologous to known protein allergens or toxins present in the PIR, SwissProt, GenPept and EMBL protein databases. There was no evidence of toxicity to mice due to the acute oral delivery of mEPSPS protein at a high dose of 45.6 mg mEPSPS protein per kg body weight, which represents a several thousand-fold safety factor over the consumption level (exposure) in food products potentially containing mEPSPS protein. These data, in combination with seed compositional analysis confirming substantial equivalence and lack of pleiotropy; 90 day rat sub-chronic toxicity testing of corn grain, and animal feeding studies confirming nutritional equivalence, support the conclusion that Roundup Ready® corn is as safe and nutritious as traditional corn varieties.

1958 CHARACTERIZATION OF *BACILLUS THURINGIENSIS* CRY2AB2 PROTEIN PRODUCED IN BT AND INSECT PROTECTED CORN AND COTTON PLANTS.

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Corn (*Zea mays*) and cotton (*Gossypium hirsutum*) plants protected from damage by lepidopteran pests are under development by Monsanto. Insect protection was accomplished by the stable insertion of a gene encoding the Cry2Ab2 protein from *Bacillus thuringiensis* (B.t.). The produced Cry2Ab2 protein protects corn and cotton from a variety of pests including black cutworm, corn earworm (CEW), south western cornborer, European cornborer, fall armyworm, tobacco budworm, pink bollworm, cotton bollworm and black army worm. These insect protected plants will benefit growers and the environment by reducing chemical insecticide usage and provide a more effective method of control of insect pests. Prior to commercialization of these varieties of corn and cotton producing the Cry2Ab2 protein, studies were conducted using purified Cry2Ab2 protein. Due to the low level of production of Cry2Ab2 protein in insect protected plants, it was not feasible to isolate Cry2Ab2 protein directly from plants. Thus bioactive protein was produced by large scale fermentation of B.t. and studies were performed to verify physical and functional equivalence to the plant-expressed protein. Gram quantities of Cry2Ab2 protein was isolated and characterized with respect to identity, purity, functional activity and composition. The corn- and cotton-produced Cry2Ab2 protein was isolated at sub-milligram levels using immunoaffinity chromatography. The equivalence of the B.t.- and plant-produced proteins was assessed. Both had comparable molecular weights, immunoreactivities and expected N-terminal amino acid sequences. Neither protein was glycosylated. Bioactivity was comparable to historical bioactivity data obtained for CEW. These data established the equivalence of the B.t.- and plant-produced proteins and served to justify the use of the B.t.-produced protein in subsequent safety studies used to support this technology.

1959 CHARACTERIZATION OF *BACILLUS THURINGIENSIS* CRY3BB1 PROTEIN PRODUCED IN B.T. AND INSECT PROTECTED CORN PLANTS.

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Corn plants (*Zea mays* L.) protected from damage by the coleopteran pest, corn rootworm (CRW), are under development by Monsanto. Insect protection was accomplished by the stable insertion of a gene encoding the Cry3Bb1 protein from *Bacillus thuringiensis* (B.t.). CRW protected corn plants will benefit corn growers and the environment by reducing chemical insecticide usage in corn and provide a more effective method of control of CRW. Prior to commercialization of CRW protected corn varieties producing the Cry3Bb1 protein, studies were conducted using purified Cry3Bb1 protein. Due to the low level of production of Cry3Bb1 protein in CRW protected corn plants, it was not feasible to isolate Cry3Bb1 protein directly from plants. Thus bioactive protein was produced by fermentation of B.t. and studies were performed to verify physical and functional equivalence to the plant-expressed protein. Gram quantities of Cry3Bb1 protein was isolated and characterized with respect to identity, purity, functional activity and composition. The corn-produced Cry3Bb1 protein was isolated at sub-milligram levels using immunoaffinity chromatography. The equivalence of the B.t.- and plant-produced proteins was assessed. Both had comparable molecular weights, immunoreactivities and expected N-terminal amino acid sequences. Neither protein was glycosylated. The bioactivity of the two proteins was comparable based on LC50 values obtained for Colorado Potato Beetle. These data established the equivalence of the B.t.- and plant-produced proteins and served to justify the use of the B.t.-produced protein in subsequent safety studies used to support this technology.

1960 REDUCTION OF OXIDATIVE DNA DAMAGE (COMET ASSAY) IN WHITE BLOOD CELLS BY BLACK TEA CONSUMPTION IN SMOKERS AND NONSMOKERS.

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Oxidative DNA damage can occur in humans following exposure to endogenous and exogenous agents, and may lead to gene mutations, conformational changes in chromosomes and modulation of gene expression, events that have been associated