Toxicity of Hydrolysis Products from 3-Butenyl Glucosinolate in Rats

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While glucosinolate breakdown products occur only in trace quantities in rapeseed meal from European and North American oil mills, glucosinolate hydrolysis during processing in small South Asian oil mills is very significant. This paper reports a study of the toxicity of breakdown products from 3-butenyl glucosinolate, the major glucosinolate in South Asian rapeseed varieties. The compounds were administered to rats by stomach intubation or intraperitoneal injection. 1-Cyano-3,4-epithiobutane was found to have severe effects on the animals. No toxic effects were registered after the administration of its polymerized form. 4-Pentenenitrile had very little effect on the growth rate of rats, and even at very high doses (600 mg/kg of body weight), no mortalities were recorded. The results from this study support the notion of farmers in South Asian countries that rapeseed cake from traditional oil-expelling processes, during which a large proportion of the glucosinolates are enzymically hydrolyzed, have a higher feed value compared with expellers cake, which contains mainly intact glucosinolates.

INTRODUCTION

Rapeseed processing in European and North American oil mills includes a heat treatment process to inactivate the glucosinolate-hydrolyzing enzyme myrosinase, thus ensuring that nearly all the glucosinolates remain intact in the oil-extracted meals. In contrast, no intentional heat treatment is practiced in small and medium-sized mills in South Asia with the consequence that a large proportion of the glucosinolates is hydrolyzed during processing, contaminating meal as well as the extracted oil.

The dominant glucosinolate of South Asian rapeseed is 3-butenyl glucosinolate, which on enzymic hydrolysis yields 3-butenyl isothiocyanate, 1-cyano-3,4-epithiobutane, and traces of 4-pentenenitrile (Dietz and King, 1987). Under comparable conditions (pH 5.5–6), 2-hydroxy-3-butenyl glucosinolate, which is the dominant glucosinolate in most European commercial rapeseed varieties, yields 3-hydroxy-4-pentenenitrile, 5-oxazolidone-2-thione, and 1-cyano-2-hydroxy-3,4-epithiobutane (*threo/erthyro*) in approximately equal proportions.

Epithionitriles tend to polymerize in polar conditions (Luthy and Benn, 1979), and it is likely that this reaction occurs at the epithio group, following a mode similar to the polymerization of epoxides (Alcock and Lampe, 1981). Very little seems to be known about any toxic effects of polymers from epithionitriles. The physiological effects of numerous saturated and unsaturated alkenylnitriles have been reported (Ahmed and Farooqui, 1982), but no studies are known to the authors which describe the toxicity of 4-pentenenitrile.

Intact glucosinolates are believed to be nontoxic (Bille et al., 1983). When ingested, however, they can be hydrolyzed to form toxic products by a myrosinase-like activity, endogenous to the bacterial microflora of the gastrointestinal tract of animals (Tani et al., 1974).

The aim of this study was to investigate the impact of small-scale rapeseed and mustard seed processing on the nutritional value of cake and oil by assessing the toxicity of the individual breakdown products from 3-butenyl glucosinolates.

MATERIALS AND METHODS

Synthesis of 4-Pentenenitrile. Bromobutene (100 mmol, 13.5 g) (Aldrich 17455-13-9), 7 mmol (2 g) of 18-crown-6 (Aldrich 18 665-1), and 415 mmol (26 g) of dry potassium cyanide were weighed into a dry 250-mL round-bottom flask. Dry acetonitrile (100 mL) was added, and the solution was stirred vigorously under gentle reflux (ca. 90 °C in an oil bath) for 36 h (Cook et al., 1974). The solids were filtered and most of the acetonitrile distilled off by using a 15-cm vigreux column. The mixture was taken up in diethyl ether and washed twice with saturated NaCl solution. The ether was dried, filtered, and evaporated. The residue was distilled under vacuum (60 °C/40 mmHg) to yield ca. 70 mmol of 4-pentenenitrile. The identity and purity of the compound were confirmed by GC-MS.

Synthesis of 1-Cyano-3,4-epithiobutane. The procedure essentially followed the method suggested by Luthy and Benn (1980).

4-Pentenenitrile (100 mmol, 8.1 g) was weighed into a 2-neck 250-mL round-bottom flask and 100 mL of dry dichloromethane added, and the flask was placed into an ice bath on a magnetic stirrer. *m*-Chloroperbenzoic acid (120 mmol, 26 g) (Aldrich C 6270-0) was dissolved in dichloromethane and added slowly from a dropping funnel to the stirred cold solution of pentenenitrile. After 1 h, the flask was taken out of the ice bath and heated to gentle reflux for 3 h and then cooled overnight to 18 °C (Hall et al., 1971). The reaction solution was further cooled to 5 °C for 30 min to precipitate chloroperbenzoic acid and then filtered. The filtrate was washed with 50 mL of sodium bisulfite solution and twice with 50 mL of saturated sodium bicarbonate solution. The solvent was dried, filtered and evaporated. The residue was distilled at 100 °C/20 mmHg.

3,4-Epoxybutanenitrile (100 mmol) was added dropwise to a stirred mixture of 100 mmol (7.6 g) of thiourea and 100 mmol (12.2 g) of benzoic acid in 100 mL of acetone at ambient temperature. After a further 5 h of stirring, a crystalline precipitate formed which was separated by filtration (Bordwell and Andersen, 1953). Without further purification 10 g of the crystals was dissolved in acetone–water (200 mL, 3:7), and sodium carbonate (20%) in water was added slowly. The pH increased to 8–9 but fell back quickly to 7. After 30 min when ca. 10 mL of the carbonate solution had been added, the aqueous mixture was extracted with benzene (20 mL). The aqueous phase was treated with more carbonate solution (ca. 5 mL) and again extracted with benzene. Finally, enough carbonate solution was added to the solution to keep the pH at 8–9. This was again

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Table I. Mortality, Weight Gain, Food Intake, and Physical Symptoms during 8 Days after the First Administration of Test Compounds

test compd	dose, mg/kg of body wt	wt gain, g	feed intake, g	feed conversion, g/g	mortality
CEB	SI 60	70.7 ± 6.2 A,B[a]	157	2.22	0/3
	SI 180	16	87	2.38	2/3
	IP 12	66.3 ± 4.7 A	165	2.49	0/3
	IP 36	47.3 ± 4.5 C	127	2.68	0/3
P-CEB	SI 60	77.3 ± 3.3 B	179	2.30	0/3
	SI 180	74.0 ± 1.6 B	174	2.36	0/3
	IP 12	72.7 ± 1.0 A,B	169	2.33	0/3
	IP 36	70.7 ± 5.6 A,B	162	2.29	0/3
4-PN	SI 60	71.3 ± 2.5 A,B	172	2.42	0/3
	SI 180	66.3 ± 2.1 A	158	2.38	0/3
	IP 12	72.0 ± 7.8 A,B	172	2.38	0/3
	IP 36	68.9 ± 5.4 A	167	2.43	0/3
control		72.8 ± 6.2 A,B	169 ± 1.8	2.32	0/12

[a] Test groups with the same letter showed no statistically significant difference.

Table II. Mortality, Weight Gain, Food Intake, and Physical Symptoms of Rats after Second Administration of Test Compounds

test compd	dose, mg/kg of body wt	wt, gain g	feed intake, g	feed conversion, g/g	mortality
CEB	SI 120	60.7 ± 3.3A	165	2.72	0/3
	SI 360				1/1
	IP 24	45.5 ± 0.5	153	2.49	1/3
	IP 72	−2.7 ± 8.0	149	16.57	2/3
P-CEB	SI 120	58.0 ± 0.0A	172	2.96	0/3
	SI 360	54.3 ± 2.6A	166	3.05	0/3
	IP 24	59.7 ± 2.1A	164	2.76	0/3
	IP 72	52.0 ± 2.5A	150	2.88	0/3
4-PN	SI 120	50.0 ± 3.6A	150	3.00	0/3
	SI 360	51.7 ± 1.7A	149	2.88	0/3
	IP 24	54.3 ± 7.1A	161	2.97	0/3
	IP 72	52.7 ± 1.7A	157	2.99	0/3
control		54.8 ± 5.7A	169 ± 1.8	3.08	0/12

[a] Test groups with the same letter showed no statistically significant difference.

extracted with benzene. The combined benzene extracts were washed with water and dried, and the solvent was evaporated under reduced pressure at room temperature.

The residual oil, ca. 5 g, was quickly distilled under reduced pressure (60 °C, 0.1 mmHg) to afford a colorless oil (3.2 g). Its identity and purity were confirmed by GC–MS.

Polymerization of 1-Cyano-3,4-epithiobutane. The epithionitrile was dissolved at the appropriate concentration in 400 μL of an aqueous solution of 5% Tween 80 (polyoxyethylene sorbitan monooleate), containing 0.9% sodium chloride. The solution was heated to boiling and then left to cool. Insoluble flakes of a light brown color formed during boiling and subsequent cooling.

Experimental Design and Conduct. Male Sprague-Dawley weanling rats (120–140 g) were housed three to a cage and given feed and water ad libitum. The experimental room was maintained at 22 °C and 40% relative humidity, with equal periods of light and darkness. Three compounds were studied: 4-pentenenitrile (4-PN), 1-cyano-3,4-epithiobutane (CEB), and its polymerized form (P-CEB). The compounds were dissolved at the appropriate concentration in 400 μL of a 0.9% sodium chloride solution containing 5% Tween 80.

Experiment 1. This was conducted in two phases. In phase 1, groups of three rats were administered with the test compounds by stomach intubation (SI) at 60 or 180 mg/kg of body weight or by intraperitoneal injection (IP) at 12 and 36 mg/kg of body weight. Four groups of three rats were given the carrier solution. The volume of each administration was 400 μL. In phase 2, carried out 8 days later, rats were administered with their respective compounds at twice the initial dose. The experiment was terminated after a further 8 days.

Experiment 2. Two groups of three rats that were given the carrier solution in phase 1 of experiment 1 were administered with 4-PN or P-CEB by IP at 200 mg/kg of body weight. Another three groups of previously untreated rats were administered high doses of the test compounds as follows: 4-PN at 600 mg/kg of body weight to two rats by SI and CEB at 600 or 200 mg/kg of body weight to three rats by SI and IP, respectively. The experiment was terminated after 8 days.

Post-Mortem Examination. All rats were sacrificed by anaesthetizing them in chloroform. Their livers, kidneys, and spleens were weighed before being fixed in buffered neutral formalin for histopathological examination.

Statistical Analysis. The growth data were analyzed by using the statistical program SAS (SAS, 1982). The analysis of variance for weight gain and organ weight was conducted by the general linear model procedure, nesting dose into treatment and application.

RESULTS AND DISCUSSION

Experiment 1, Phase 1. Effects on rats after first administration of test compounds are shown in Table I.

The most severe toxic effects occurred in rats that were administered CEB. When given by SI at 180 mg/kg of body weight, two of the three rats died between 3 and 18 h after administration, while the surviving animal expressed strong vocalization to touch, had lost its righting reflex, its muscle tone was reduced considerably, its general activity was low, and weight gain and food intake were lower than control values. At the lower dose (60 mg/kg of body weight) weight retardation in comparison with the control group was not statistically significant. Application of CEB by IP at 20% of the SI dose resulted in a reduced weight gain compared with controls, the differences being statistically significant at the higher dose ($P < 0.05$).

P-CEB did not affect growth rate at any of the doses or administration methods used. Weight gain was, in fact, higher than that in the control group, but the differences were not statistically significant. The physical condition and behavior of the rats were also normal, suggesting that the animals had not been affected by P-CEB.

Administration of the high dose of 4-PN by SI and IP caused a significant reduction in weight gain compared with rats given P-CEB. The differences in comparison with the control animals were, however, not statistically significant. Twenty hours after administration of 4-PN by SI, the activity of rats was reduced and they were less alert than the control group.

None of the test compounds affected the feed conversion ratio.

Experiment 1, Phase 2. Effects on rats after administration of the second dose of test compounds are shown in Table II.

For the second part of experiment 1 the concentration of nitriles administered was doubled. CEB dosed by SI at 120 mg/kg caused no adverse effects on food intake and weight gain. However, the general activity of these rats was low for approximately 20 h, they were very sensitive to touch, and their righting reflex and muscle tone were reduced. Administration of CEB by SI at 360 mg/kg of body weight to the animal that had survived the first dosing resulted in its death between 3 and 18 h after the application. The weight gain of rats given the low and high doses of CEB by IP was significantly reduced ($P < 0.05$). One rat in the low-dose group and two in the high-dose group died between 27 and 40 h after application. At both dose levels, the general activity, muscle tone, and righting reflexes of rats were considerably reduced for 36 h after application. The weight gain of rats dosed with 4-PN and P-CEB by SI or IP was not significantly affected

Table III. Mortality, Physical Condition, Weight Gain, and Food Intake of Rats during 8 Days after Administration of High Doses of Test Compounds

test compd	dose, mg/kg of body wt	wt gain, g	feed intake, g	feed conversion, g/g	mortality
4-PN	SI 600	51.7 ± 8.50	155	3.01	0/2
	IP 200	57.3 ± 2.62	166	2.91	0/3
P-CEB	IP 200	51.7 ± 1.70	168	3.26	0/3
CEB	SI 600	4.3	54	12.56	2/3
	IP 200	*a*	*a*	*a*	3/3

[a] All animals died shortly after administration of the test compounds.

in comparison with the controls. However, in both groups the general activity of rats at the higher dose level was low, and the animals were slightly sensitive to touch 20 h after application.

Experiment 2. The effects on rats after administration of high doses of the test compounds are shown in Table III.

As in experiment 1, 4-PN and P-CEB did not produce any mortality or depression of the growth rate, but CEB had a severe detrimental effect on rats. All three rats given CEB at 200 mg/kg of body weight by IP, and two of the three rats given CEB at 600 mg/kg of body weight by SI died between 3 and 18 h after administration.

Administration of 4-PN at 600 mg/kg of body weight by SI and at 200 mg/kg of body weight by IP produced involuntary tremors and minor convulsions in rats within a few minutes, whereas CEB produced depression. 4-PN also produced diarrhea in rats 24 h after administration.

Organ Weights of Rats. Rats that had died in the course of the experiment were not included in the statistical analysis of the relative organ weights because several hours may have passed between death and post-mortem examination, possibly resulting in autolysis of the liver, kidney, and spleen. Rats in all three test groups appeared to have enlarged livers when the figures were expressed relative to body weight (Table IV). Similarly, the relative kidney weights of rats administered with high doses of CEB by SI or IP were also significantly increased. The significance of these observations is, however, not clear in view of the fact that body weights of some rats had reduced during what was a short experimental period.

Histopathological Assessment. The sole tissues to demonstrate significant pathological change were the livers from rats dosed with CEB. Two of the three rats given a single dose of CEB by SI at 600 mg/kg of body weight had mild to moderate periacinar necrosis and congestion. Terminal autolysis of the organs in the third rat precluded adequate assessment of its organs. All three rats given a single dose of CEB by IP at 200 mg/kg of body weight also had minimal to mild periacinar necrosis and mild to moderate periacinar congestion.

The kidney of a rat that died as a result of being administered CEB by SI at 180 followed by 360 mg/kg of body weight had a possible pattern of tubular degeneration which, however, may have been a result of terminal hypoxia rather than a specific effect.

The results of this investigation confirm the acute toxicity of epithionitriles. Administration of a single dose of CEB by SI at 180 mg/kg of body weight, a previously reported LD_{50} dose (Tookey et al., 1980), resulted in the death of two of three animals within 20 h, although no ill effects were observed at 30% of this dose. Administration of a single dose of CEB by IP at 36 mg/kg of body weight did not produce any deaths during 8 days, but had a considerable adverse effect on weight gain, while at 200 mg/kg of body weight all three dosed rats died. Histopathological assessment of the livers showed mild to moderate periacinar necrosis and congestion.

The toxicity of epithionitriles is probably caused by ring-opening reactions with nucleophiles, similar to the mechanism of action of oxiranes, with the consequential toxic effects of an alkylating agent. Brocker et al. (1984) reported that the main metabolic route of epithionitriles was its conjugation to glutathione via nucleophilic ring opening in the epithio group, followed by excretion as mercapturic acid. Structurally related groups, such as oxiranes, undergo similar reactions which may suggest that thiiranes, like these compounds, are carcinogens (Druckrey et al., 1970). Results from in vitro tests have suggested that epithionitriles are weak mutagens (Luthy and Benn, 1980).

The effects of epithionitrile-containing diets have been reported by numerous authors. Gould et al. (1980) described the pathological changes associated with feeding of 1-cyano-2-hydroxy-3,4-epithiobutane to rats over 90 days. Body weights were significantly reduced at 75 mg of epithionitrile/kg of diet in comparison with a control group, while the weights of liver and spleen appeared to be significantly increased at a level of 300 mg/kg of diet. Liver and kidney lesions were already present at the concentration of 75 mg/kg of diet and increased in severity and incidence at the 150 and 300 mg/kg levels. The lesions observed were similar to those seen after ingestion of aflatoxins and pyrrolizidine. Such lesions were, however, not observed in the experiments reported here. Hepatocellular damage was indicated also by the elevations of certain enzymes. No clinical signs of acute or chronic cyanide intoxication were found in the epithionitrile-treated animals.

The practical importance of epithionitrile toxicity may, however, be limited. Epithionitriles are unstable in polar matrices and tend to polymerize. The concentration of epithionitriles decreases rapidly in rapeseed and mustard seed meals or press cakes. In general, they cannot be found in dichloromethane extracts prepared from press cakes 24 h after enzymic hydrolysis (Dietz and Harris, unpublished data). Ingestion of meals or press cakes containing intact glucosinolates and active myrosinase may result in the formation of epithionitriles. However, the epithiospecifier protein, the cofactor responsible for the rearrangement of the unstable intermediate from the enzymic hydrolysis, is very labile, and oilseed processing methods that only partially inactivate the myrosinase will almost certainly inactivate the epithiospecifier protein. Glucosinolate hydrolysis may also occur by the catalytic activity of bacterial myrosinase in the gastrointestinal tract. However, there have been no reports of the presence of an epithiospecifier activity associated with bacterial myrosinase, which is required for the rearrangement to take place.

The absence of major adverse effects of P-CEB, even at high doses, on the growth of rats and on the histological structure of the major metabolic organs, when administered by the SI or the IP route during this study, suggests that it may be of relatively nontoxic nature.

4-Pentenenitrile is formed from 3-butenyl glucosinolate only in trace quantities at pH 5–6.5, which is the normal pH range for moist rapeseed meal (Dietz and Harris, unpublished data). In the present study, administration of 4-PN by the SI or IP route had no significant effects on the growth rate of rats, and even at very high doses no mortality was recorded.

Reports on the toxicity of the structurally related

Table IV. Relative Organ Weights of Animals

test compd	dose, mg/kg of body wt	liver wt, g/100 g of body wt	kidney wt, g/100 g of body wt	spleen wt, g/100 g of body wt
CEB	SI 60/120	5.33 ± 0.14 A[a]	0.87 ± 0.03 A[a]	0.30 ± 0.04 A[a]
	SI 180/360	5.39 ± 0.35[b]	1.07 ± 0.12[b]	0.34 ± 0.06[b]
	IP 12/24	5.35 ± 0.58	0.86 ± 0.14	0.32 ± 0.05
	IP 36/72	5.42 ± 0.25	1.12 ± 0.08	0.36 ± 0.03
CEB	SI 600	6.56 ± 0.43	1.31 ± 0.14	0.26 ± 0.05
	IP 200	5.93 ± 0.52	1.09 ± 0.09	0.22 ± 0.03
P-CEB	SI 60/120	5.58 ± 0.59 A	0.85 ± 0.09 A	0.30 ± 0.02 A
	SI 180/360	5.21 ± 0.39 A	0.81 ± 0.07 A	0.32 ± 0.05 A
	IP 12/24	5.32 ± 0.40 A	0.79 ± 0.04 A	0.33 ± 0.03 A
	IP 36/72	5.03 ± 0.17 A	0.82 ± 0.08 A	0.42 ± 0.05 B
P-CEB	IP 200	5.37 ± 0.10 A	0.88 ± 0.03 A	0.35 ± 0.03 AB
4-PN	SI 60/120	4.95 ± 0.41 A	0.84 ± 0.08 A	0.29 ± 0.03 A
	SI 180/360	5.67 ± 0.53 A	0.84 ± 0.05 A	0.32 ± 0.04 A
	IP 12/24	5.65 ± 0.44 A	0.91 ± 0.12 A	0.32 ± 0.01 A
	IP 36/72	5.27 ± 0.41 A	0.85 ± 0.04 A	0.29 ± 0.02 A
4-PN	SI 600	5.23 ± 0.05 A	0.91 ± 0.01 A	0.38 ± 0.01 B
	IP 200	5.19 ± 0.32 A	0.81 ± 0.04 A	0.34 ± 0.03 AB
control	SI	4.83 ± 0.40 A	0.80 ± 0.02 A	0.33 ± 0.04 A
	IP	4.91 ± 0.09 A	0.85 ± 0.04 A	0.34 ± 0.03 A

[a] Test groups with the same letter showed no statistically significant difference. [b] Test groups without letter were not included in the statistical analysis because animals had died during the experiment at times when it was not possible to recover the organs immediately for weighing.

compound 3-hydroxy-4-pentenenitrile, a hydrolysis product from 2-hydroxy-3-butenyl glucosinolate, are contradictory.

Nishi and Daxenbichler (1980) reported that the LD_{50} of 3-hydroxy-4-pentenenitrile was 200 mg/kg of body weight when administered by SI, with deaths being preceded by a loss of righting reflex and intermittent rolling seizures. No indication was given about the preparation and purity of the test compound. Srivastava et al. (1975) reported that all chicks fed a diet containing 3.9 mg/g 3-hydroxy-4-pentenenitrile died within 7 days. A diet containing 2 mg/g 3-hydroxy-4-pentenenitrile resulted in the death of all rats within 2 weeks (VanEtten et al., 1969). These experiments were carried out with rapeseed or crambe diets that contained a combination of glucosinolate hydrolysis products. The diets were prepared fresh daily, and the epithionitrile could therefore have been present in the monomeric form.

In contrast, Slominski et al. (1984) did not observe any toxic effects when feeding a crude extract of 3-hydroxy-4-pentenenitrile at 0.6 mg/g of diet to rats. Cansfield and Campbell (1980) recorded no detrimental effects when chicks were fed for 56 days on a diet containing 0.8 mg/g 3-hydroxy-4-pentenenitrile. The occurrence of liver hemorrhage in the birds was attributed by the authors to the presence of intact 2-hydroxy-3-butenyl glucosinolate rather than to the unsaturated nitrile.

Major differences have been reported for the toxicity of different nitriles. Zeller et al. (1969) found an LD_{50} of 5000 mg/kg of body weight for 1-cyano-2-hydroxyethane, a compound similar to 3-hydroxy-4-pentenenitrile but lacking the unsaturation. In contrast, propionitrile was reported to have an LD_{50} of 40 mg/kg in rats when given orally (Ahmed and Farooqui, 1982), while its hydroxylated form, β-hydroxypropionitrile, was reported to be nontoxic (Zeller et al., 1969).

Whereas saturated nitriles, such as butyronitrile and malononitrile, produced symptoms in animals resembling inorganic cyanide poisoning showing central nervous system effects, unsaturated nitriles released less cyanide and signs of their toxicity were described as cholinomimetic, such as salivation, vasodilation, and diarrhea (Ahmed and Farooqui, 1982).

It appears that the presence of unsaturated bonds and functional groups in the chemical structure has an important bearing on toxicity of the nitriles and changes their action mechanism. Even high concentrations of aliphatic unsaturated nitriles failed to produce detectable inhibitory effects on cytochrome *c* oxidase, a typical sign for the presence of cyanide.

Wallig et al. (1988) compared the toxicity of valeronitrile, which has the same chemical structure as 4-pentenenitrile except that it lacks the unsaturation, with that of 1-cyano-3,4-epithiobutane. Rats treated with 175 mg/kg valeronitrile had acute aliphatic nitrile intoxication, which expressed itself as hypersalivation, mild diarrhea, and intermittent short tonic-clonic seizures. Similar symptoms were recorded after the administration of 4-pentenenitrile in the experiments reported here. No consistent gross alteration in the animals' organs were observed by Wallig's group.

3-Butenyl isothiocyanate which is also formed during the enzymic hydrolysis of 3-butenyl glucosinolate, was not included in the experiments. Earlier studies, however, have shown that this volatile compound (bp 58 °C) is lost almost completely during processing and only traces can be found in the cake (Dietz, 1987).

Conclusions. Rapeseed processing in Europe and North America emphasizes the importance of myrosinase inactivation during processing to avoid poisoning the nickel catalyst by sulfur contaminations in the oil and to improve the feed value of the press cake. In contrast, traditional processing of *Brassica* oilseeds in South Asia potentiates the action of myrosinase to achieve a maximum hydrolysis of glucosinolates. Results from the study presented here seem to give support to the experience of farmers that traditional processing methods during which glucosinolates are hydrolyzed are an effective way of increasing the feed value of the oilseed cake.

Most of the glucosinolates in South Asian rapeseed and mustard seed are present as nonhydroxylated alkenyl glucosinolates such as 2-propenyl and 3-butenyl glucosinotes. Epithionitriles from the enzymic hydrolysis are unstable in the press cake and will polymerize during and after processing. Animals dosed with the polymerized form did not show any toxic effects during this study. 4-Pentenenitrile is formed only in very small quantities from 3-butenyl glucosinolates in the pH range normally found in ground, moist rapeseed. But even at very high concentrations, this compound had no significant impact on the growth rate of the test animals and did not result in any mortalities. 2-Propenyl and 3-butenyl glucosino-

lates only yield volatile isothiocyanates, which are largely removed during processing. Such isothiocyanates do not cyclize to form nonvolatile oxazolidonethiones. Rapeseed and mustard seed cake prepared by methods during which glucosinolates are hydrolyzed may therefore have a potential use in nonruminant livestock rations. More research is required to assess whether polymers formed from epithionitriles in the press cake are similar in their physiological effect to those prepared from synthesized epithionitriles during this experiment. This work has been carried out and will be published shortly. Furthermore, feeding studies will be necessary to assess the long-term effects of feeds prepared by such methods.

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Registry No. 4-Pentenenitrile, 592-51-8; bromobutene, 92221-74-4; acetonitrile, 75-05-8; 1-cyano-3,4-epithiobutane, 54096-45-6; 1-cyano-3,4-epithiobutane, polymer, 130146-82-6; 3,4-epoxybutanenitrile, 30491-94-2.