Permit#	04-009-01r
Company:	Agricultural Research Service
Organism	Indian Mustard (Brassica juncea
Category	Phytoremediation
Transgene	SMT sulfurylase
1. Confinement	
Confinement and mitigation conditions have been reviewed and determined to be adequate	Х
2. Threatened or Endangered Species or its habitat	
resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
resident or migratory in counties and harm to threatened or endangered species is unlikely	
none observed in area (no harm to threatened and endangered species)	Х
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X3
Not new and prior EA	
4. New or Novel Trait (gene product)	
Never used in a field trial	Х
Not new but no prior EA	
Not new and prior EA	
Raises new issues 5. Cumulative Effects	
Cumulative Effects likely	
Cumulative effects possible	
Cumulative effects unlikely	Х
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wind pollinated food or feed crop	
Primarily Self fertilized food or feed crop	Х
Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	
food Safety not established	X
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	000 4000 K ⁸
AOSCA standard for crop	600, 1320 ft ³
Proposed isolation distance	1500 ft
9. Scale	
>100 acres/trait/crop/company/year	
50-99 acressmancrop/company/year	
10-49 acressmancrop/company/year	Y
<10 acrestrativiciopicompany/year	X
Similar affarts expected/observed	
Minimal on-cumulative effects expected/observed	
No affects expected/observed	X
11 Sexually Compatible Relatives	X
relatives within diseased distance	
relatives not within dispersal distance	X
12. Seed Dormancy	
-3 years	X ¹²
3 years	
2 years	
<2 years	
13. Persistence in environment	
Crop can naturalize	X ¹³
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	
14. Comments	

³EA completed for other related Brassica species.

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively.

¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic plants will not be allowed to produce seed.

¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Permit#	04-009-01r
Company:	Agricultural Research Serv
Organism	Indian Mustard (Brassica ju
Category	Phytoremediation
Transgene	ATP sulfurylase
1. Continement	×
Commentent and mugation condutions have been reviewed and determined to be adequate	A
2. Threatened of Endangered Species of its habitat	
resident or migratory in counties and harm to threatened or endangered species or habitar is likely	
Testion of migratory in counters and material and	X
New or Novel	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X ³
Not new and prior EA	
4. New or Novel Trait (gene product)	- · · ·
Never used in a field trial	
Not new but no prior EA	X
Not new and prior EA	
Raises new issues	
5. Cumulative Effects	
Cumulative Effects likely	
Cumulative effects possible	
Cumulative effects unlikely	X
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wind pollinated food or feed crop	
Primarily Self fertilized food or feed crop	Х
Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	
food Safety not established	X
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AUSCA standard for crop	600, 1320 ft
Proposed Isolation distance	1500 ft
9. Scale	
>100 acres/trait/crop/company/year	
50-59 acres/mai/crop/company/year	
10-49 acres/nai/crop/company/gear	X
 a destraincoprompany/year 10 Effects (procision and processing) on other species 	^
Similar affarts experted/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	X
11 Sexually Compatible Relatives	^
relatives within dispersal distance	
relatives not within dispersal distance	X
12. Seed Dormancy	
>3 vers	X ¹²
3 years	
2 years	
<2 years	
13. Persistence in environment	
Crop can naturalize	X ¹³
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	
14. Comments	
³ EA completed for other related Brassica species.	

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively. ¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic plants will not be allowed to produce seed. ¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Dermit#	04-009-01r
Company.	Agricultural Research Service
Orranism	Indian Mustard (Brassica juncea)
Cateoory	Phytoremediation
Transgene	Cytosolic selenocystine lyase
· · ·	
1. Confinement	X
Continement and mitigation conditions have been reviewed and determined to be adequate	X
2. Infreatened or Enclangered Species or its habitat	
resident or migratory in counties and harm to threatened or endangered species or habital is likely	
none observed in area (no harm to threatened and endangered species)	X
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X ³
Not new and prior EA	
4. New or Novel Trait (gene product)	
Never used in a field trial	X
Not new but no prior EA	
Not new and prior EA	
Raises new issues	
or cumulative Effects	
	┨─────┤
	×
6 Plant Pollingtion	^
Primarily Bee or insect pollinated crop	
Primarily Wind polinated food or feed crop	
Primarily Self fertilized food or feed crop	Х
Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	
food Safety not established	Х
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	000 4000 # 8
AUSCA standard for crop Demonstrational isolations	600, 1320 It
	1500 lt
s. state	1
5 food acres/trait/crop/company/year	
10-49 acres/trait/cprod/company/year	
<10 acres/trait/crop/company/vear	X
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	X
11. Sexually Compatible Relatives	1
relatives within dispersal distance	· · · · · · · · · · · · · · · · · · ·
relatives not within dispersal distance	×
	X ¹²
3 years	~
2 years	
<2 years	
13. Persistence in environment	
Crop can naturalize	X ¹³
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	
14. Comments	
"EA completed for other related Brassica species.	
AUSCA does not have a standard for <i>B. juncea</i> . The standard for <i>B napus</i> and <i>B. rapa</i> is 660 and 1320 ft,	
respectively.	
Data for <i>B. juncea</i> not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic	
prants will not be allowed to produce seed. ¹³ Sevually compatible species will be removed from within 1500 feet of the text area and plants will be beneated prior.	
to seed production.	

Additional supporting documentation is found in the summary risk assessment completed on

Permit#	04-009-01r
Company:	Agricultural Research Service
	Indian Mustard (Brassica junces
	Dhuteremediation
	Phytoremediation
Iransgene	Chloroplastic selenocystine lyas
1. Confinement	
Confinement and mitigation conditions have been reviewed and determined to be adequate	X
2. Threatened or Endangered Species or its habitat	
resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
resident or migratory in counties and harm to threatened or endangered species is unlikely	
none observed in area (no harm to threatened and endangered species)	Х
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior FA	X3
Not new and prior EA	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4. New of Novel Trail (gene product)	X
Never used in a field trial	X
Not new but no prior EA	
Not new and prior EA	
Raises new issues 5. Cumulative Effects	
Cumulative effects possible	
Cumulative effects unlikely	X
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wind pollinated food or feed crop	
Primarily Self fertilized food or feed crop	Х
Non-food or feed crop	
7. Effects on Food/Feed Supply	•
Known allergen, antinutrative, oral toxicant	
food Safety not established	Х
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
S Isolation Distance	
AOSCA standard for crop	600_1320 ft ⁸
	1500 #
	1500 It
9. Scale	
>100 acres/trait/crop/company/year	
50-99 acres/trait/crop/company/year	
10-49 acres/trait/crop/company/year	
<10 acres/trait/crop/company/year	Х
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	Х
11. Sexually Compatible Relatives	
relatives within dispersal distance	
relatives not within dispersal distance	X
12. Seed Dormancy	· · · · · · · · · · · · · · · · · · ·
	X ¹²
3 years	
<2 years	
13. Persistence in environment	
Crop can naturalize	X'3
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	
14. Comments	
³ EA completed for other related Brassica species.	

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively. ¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic plants will not be allowed to produce seed.

¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Permit# Company: Organism	04-009-01r Agricultural Research Service Indian Mustard (Brassica juncea)
	Cystathiopine gamma synthase
	Cystatilionine gamma synthase
1. Confinement	
Confinement and mitigation conditions have been reviewed and determined to be adequate	Х
2. Threatened or Endancered Species or its habitat	
resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
resident or migratory in counties and harm to threatened or endangered species is unlikely	
none observed in area (no harm to threatened and endangered species)	Х
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X ³
Not new and prior EA	
4. New or Novel Trait (gene product)	
Never used in a field trial	Х
Not new but no prior EA	
Not new and prior EA	
Raises new issues	
5. Cumulative Effects	
Cumulative Effects likely	
Cumulative effects possible	
Cumulative effects unlikely	Х
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wind pollinated food or feed crop	
Primarily Self fertilized food or feed crop	Х
Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	
food Safety not established	Х
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AOSCA standard for crop	600, 1320 ft ⁸
Proposed isolation distance	1500 ft
9. Scale	
>100 acres/trait/crop/company/year	
50-99 acres/trait/crop/company/year	
10-49 acres/trait/crop/company/year	
<10 acres/trait/crop/company/year	X
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Ininimal, non-cumulative effects expected/observed	
No effects expected/observed	X
11. Sexually Compatible Relatives	
relatives within dispersal distance	
relatives not within dispersal distance	X
12. Seed Dormancy	v12
>3 years	X'-
3 years	
2 years	
<2 years	
13. Persistence in environment	
Crop can naturalize	X
Crop can persist 3-5 years without numan intervention	
³ EA completed for other related Brassica spacies	
En completed for utiler related brassica species.	

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively. ¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic plants will not be allowed to produce seed. ¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Permit#	04-009-01r
Company:	Agricultural Research Service
Organism	Indian Mustard (Brassica juncea
Category	Phytoremediation
Transgene	Methionine methyltransferase
1. Continement	¥
2 Threatened or Endangered Species or its habitat	~
resident or migratory in counties and harm to threatened or endangered species or babitat is likely	
resident or migratery in counties and harm to threatened or endangered species is unlikely	
none observed in area (no harm to threatened and endangered species)	X
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X ³
Not new and prior EA	
4. New or Novel Trait (gene product)	
Never used in a field trial	X
Not new but no prior EA	
Not new and prior EA	
Raises new issues	
5. Cumulative Effects	
Cumulative Effects likely	
Cumulative effects possible	
Cumulative effects unlikely	X
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wild pointated food or food arep	×
Printany Sen refuzzed food of reed crop	^
7 Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	
fond Safety not established	X
GRAS status or approved food additive for native protein	~
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AOSCA standard for crop	600, 1320 ft ⁸
Proposed isolation distance	1500 ft
9. Scale	· · · · · ·
>100 acres/trait/crop/company/year	
50-99 acres/trait/crop/company/year	
10-49 acres/trait/crop/company/year	
<10 acres/trait/crop/company/year	Х
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	Х
11. Sexually Compatible Relatives	•
relatives within dispersal distance	
relatives not within dispersal distance	X
12. Seed Dormancy	242
>3 years	X'2
3 years	
<2 years	
42 Develotones in environment	
13. Persistence in environment	V13
13. Persistence in environment Crop can naturalize Crop can parait 3.5 years without human intervention	X ¹³
13. Persistence in environment Crop can naturalize Crop can persist 3-5 years without human intervention Crop does not persist without intervention	X ¹³
13. Persistence in environment Crop can naturalize Crop can persist 3-5 years without human intervention Crop does not persist without intervention 14. Comments	X ¹³

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively. ¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic plants will not be allowed to produce seed. ¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Permit# Company: Organism Category Transgene	04-009-01r Agricultural Research Service Indian Mustard (Brassica juncea Phytoremediation Na*H* exchanger
1. Confinement	× ×
2. Threatened or Endengated Species or its habitat	×
resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
resident or migratory in counties and harm to threatened or endangered species is unlikely	
none observed in area (no harm to threatened and endangered species)	Х
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X°
Not new and prior EA	
4. New or Novel Trait (gene product)	X
Not new but no prior FA	^
Not new and prior EA	
Raises new issues	
5. Cumulative Effects	
Cumulative Effects likely	
Cumulative effects possible	
Cumulative effects unlikely	X
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wind pollinated food or feed crop	X
Primarily Self fertilized food of feed crop	X
7 Effects on Ecod/Eeed Supply	
Known allergen, antiputrative, oral toxicant	
food Safety not established	X
GRAS status or approved food additive for native protein	<u>^</u>
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AOSCA standard for crop	600, 1320 ft ⁸
Proposed isolation distance	1500 ft
9. Scale	
>100 acres/trait/crop/company/year	
50-99 acres/trait/crop/company/year	
10-49 acres/trait/crop/company/year	
<10 acrestrative or negative) on other species	X
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	X
11. Sexually Compatible Relatives	
relatives within dispersal distance	
relatives not within dispersal distance	Х
12. Seed Dormancy	
>3 years	X ¹²
3 years	
2 years	
<2 years 12 Dersistance in environment	
ro, reisistende in environment	¥ ¹³
Crop can nersist 3-5 years without human intervention	^
Crop does not persist without intervention	
14. Comments	
³ EA completed for other related Brassica species.	

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively.
¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic plants will not be allowed to produce seed.
¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed

Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Permit#	04-009-01r
Company:	Agricultural Research Service
Organism	Indian Mustard (Brassica juncea)
Category	Phytoremediation
Transgene	gamma glutamylcysteine synthase
1. Confinement	
Confinement and mitigation conditions have been reviewed and determined to be adequate	Х
2. Threatened or Endangered Species or its habitat	
resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
resident or migratory in counties and harm to threatened or endangered species is unlikely	
none observed in area (no harm to threatened and endangered species)	Х
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X3
Not new and prior EA	
4. New or Novel Trait (gene product)	
Never used in a field trial	
Not new but no prior EA	Х
Not new and prior EA	
Raises new issues	
5. Cumulative Effects	
Cumulative Effects likely	
Cumulative effects possible	
Cumulative effects unlikely	Х
6. Plant Pollination	
Primarily Bee or insect pollinated crop	
Primarily Wind pollinated food or feed crop	
Primarily Self fertilized food or feed crop	Х
Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	

Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutrative, oral toxicant	
food Safety not established	Х
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AOSCA standard for crop	600, 1320 ft ⁸
Proposed isolation distance	1500 ft
9. Scale	
>100 acres/trait/crop/company/year	
50-99 acres/trait/crop/company/year	
10-49 acres/trait/crop/company/year	
<10 acres/trait/crop/company/year	Х
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	Х
11. Sexually Compatible Relatives	
relatives within dispersal distance	
relatives not within dispersal distance	Х
12. Seed Dormancy	
>3 years	X ¹²
3 years	
2 years	
<2 years	
13. Persistence in environment	
Crop can naturalize	X ¹³
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	
14. Comments	
VEA assumption for other related Dressing energies	

³EA completed for other related Brassica species.

⁸AOSCA does not have a standard for *B. juncea*. The standard for *B napus* and *B. rapa* is 660 and 1320 ft, respectively. ¹²Data for *B. juncea* not available, but relatives are known to have seed dormancy exceeding 5 years. The transgenic

plants will not be allowed to produce seed.

¹³ Sexually compatible species will be removed from within 1500 feet of the test area and plants will be harvested prior to seed production.

Additional supporting documentation is found in the summary risk assessment completed on

Based on a review of Permit 04-009-01r, the following determinations were made:

- The sixteen threatened and endangered species recognized by the U.S. Fish and Wildlife Services as occurring in the county where the trial will be performed will not be affected by this field release. Five are plants, six are aquatic or semi-aquatic, two will not feed on Brassica. The remainder will be excluded by enclosing plants with chicken wire fence and overhead netting.
- Field trials have been performed with transgenic Indian mustard (*Brassica juncea*) plants under APHIS authority, and APHIS is familiar with *B. juncea* biology and methods to manage confined *B. juncea* field trials.
- The outcrossing rate for *B. juncea* is similar to *B. napus*: it is two-thirds selfpollinating and one-third insect pollinating The Association of Official Seed Certifying Agencies (AOSCA) does not provide specific isolation distance recommendations for *B. juncea*, but isolation distances recommended for foundation seed production of outcrossing rapeseed (such as *B. rapa* and unspecified species of mustard) is 1320 ft, and for self-pollinating rapeseed (such as *B. napus*) is 660 ft. The Canadian Food Inspection Agency recommends an isolation distance from weedy relatives of 50 meters. The applicant proposes an isolation distance of 1500 feet not only from *B. juncea*, but all other Brassica related crops.
- Any plant material left after harvest, containing only insignificant amounts of the proteins, will be plowed under the soil surface. The proteins have no known or foreseeable toxic effects, so this method of disposal should have no negative impacts on the environment.
- The gene products proposed for these field trials do not have characteristics of known toxins or allergens. No foreseeable effects on other organisms are expected.
- The actual area to be planted with transgenic plants is 0.018 acre. Trials of such small size are and have been easily monitored and confined to permitted areas, under environmental mitigation measures similar to those specified in the permit application and in the standard and supplemental permit conditions.
- The crop will be harvested prior to setting seed to mitigate the possibility that *B*. *juncea* establishes in unmanaged environments.
- The field site will be monitored to eliminate all sexually-compatible relatives of *B. juncea* growing within 1500 feet of the trial.

For the above reasons, APHIS has determined that (1) pursuant to 7 C.F.R. §372, the field trials proposed under permit #04-009-01r will not significantly affect the physical environment and (2) there are no applicable, extraordinary, or other reasonably foreseeable circumstances under which significant environmental effects could occur given the protective and ameliorative measures specified above. Therefore, this field test is deemed confined within the meaning of 7 C.F.R. §372.5.

Signed: _____

Susan Koehler Branch Chief, Environmental and Ecological Analysis Date: ____7.19.04_____