

RESEARCH ARTICLE

Toxicity of Plant Derived Molluscicides in Attractant Food Pellets against Snail, *Lymnaea Acuminata*

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ABSTRACT

Use of molluscicides in the attractant food pellet (AFP) is one of the effective methods of snail control. Attractant food pellets containing starch and agar plus different concentrations of these molluscicides (Azadirachta indica bark powder, Allium sativum bulb powder, Polianthes tuberosa bulb powder, Annona squamosa seed powder, their active components azadirachtin, allicin, hecogenin, acetogenin; herbal molluscicide pestoban and a synthetic molluscicide, Snail Kill, were tested for molluscicidal activity for 144h against the snail, *Lymnaea acuminata*. Active components of all the plant derived molluscicides were highly toxic to *L. acuminata* compared with their crude forms. The stability of bait formulations was studied by storing the pellets up to 4 weeks. Storage of molluscicide baits caused higher reduction in their toxicity in comparison with synthetic molluscicides.

Keywords: Attractant food pellets, Bait formulation, *Fasciola*, *L. acuminata*, Molluscicides, Starch

Liver- flukes *Fasciola hepatica* Linnaeus and *Fasciola gigantica* Cobbold cause endemic fascioliasis in cattle population of eastern Uttar Pradesh [1-2]. The snail *Lymnaea* (*Radix*) *acuminata* Lamarck (*Lymnaei*-*daea*) is the vector of these flukes. One way to reduce the incidence of fascioliasis is to de-link the life cycle of fluke by destroying the intermediate hosts [3-8]. The development of a selective and safe molluscicide should always be a realistic goal. It must be effective at low concentrations and exert minimal adverse effect on the other biota sharing the same habitat with snail. Lack of contact between molluscicides and target snail population due to meshy vegetation, dilution in upwelling sewage water are two main causes of the failure of snail control programme. The snails use chemical signals for locating food sources. These signals are released from the dead and living aquatic organisms into the modular system of the snails [9-13]. Starch is the strongest attractant for *L. acuminata* [14]. Bait formulation containing attractant and a molluscicide is an expedient approach in order to lure the target snail population to the molluscicide. In the present study different plant derived molluscicides have been used along with starch in bait formulation against *L. acuminata*.

MATERIALS AND METHODS

Agar, starch, different plant derived molluscicides such as *Allium sativum* and *Polianthes tuberosa* bulb powder, *Annona squamosa* seed powder and *Azadirachta indica* bark powder and their active components allicin, hecogenin, acetogenin and azadirachtin (Sigma Chemical Co. USA), Snail Kill (metaldehyde-Pesticide India) and herbal molluscicide, Pestoban (Liquid concentrate of *Cedrus deodara*, *Azadirachta indica* and seed powder of *Embelia ribes* in 90:2:1 ratio- Indian Herbs, Research and Supply Co. Pvt. Ltd., India) were used in bait formulation. Adult *L. acuminata* (2.25±0.20 cm in length) were collected locally from lakes and low lying submerged fields in Gorakhpur. The snails were acclimatized for 72 hours in dechlorinated tap water at 25±1°C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were set to 6.5-7.2 mg/l, 5.2-6.3 mg/l and 102.0-105.0 mg/l, respectively.

Attractant food pellets (AFP) were prepared according to previous method [15] as modified by us [16]. 10 grams of starch (10 mM) was added to 2% agar solution. After boiling, each of the selective molluscicides were added to the solution in different concentrations (Table1), the mixture was stirred constantly for 30 minutes and spread to a uniform thickness (5 mm). After

Table 1. Mean number of snail *L. acuminata* in zone three in contact with the attractant food pellets (AFP) that contain different molluscicides after two hours from beginning of experiment.

Molluscicides	Concentration of molluscicides				
	0.1%*	0.2%	0.5%	0.7%	1.0%
<i>A. squamosa</i> (SP)	1.16±0.16 (58.0) +	0.83±0.16 (43.0)	1.33±0.21 (53.4)	1.67±0.21 (45.6)	0.83±0.16 (50.0)
<i>A. sativum</i> (BP)	3.67±0.2 (78.0) +	3.5±0.96 (53.8)	3.5±0.22 (61.8)	3.0±0.44 (50.0)	2.67±0.42 (47.0)
<i>P. tuberosa</i> (BP)	3.0±0.25 (60.0) +	2.67±0.21 (57.2)	2.33±0.21 (53.8)	1.83±0.40 (52.2)	1.67±0.21 (45.3)
<i>A. indica</i> (Ba P)	3.5±0.34 (60.0) +	1.83±0.16 (37.8)	3.0±0.22 (55.6)	2.33±0.42 (53.8)	0.83±0.30 (31.2)
Acetogenin	3.0±0.36 (50.0) +	2.0±0.25 (50.0)	1.16±0.3 (27.8)	1.5±0.22 (42.8)	0.5±0.22 (20.0)
Allicin	1.4±0.35 (46.7) +	1.33±0.21 (46.9)	1.0±0.63 (33.3)	1.5±0.34 (42.8)	0.83±0.16 (27.6)
Hecogenin	0.67±0.21 (50.3) +	0.33±0.21 (22.0)	0.5±0.22 (33.3)	0.83±0.30 (27.6)	0.33±0.21 (13.2)
Azadirachtin	1.16±0.16 (46.4) +	1.0±0.25 (31.5)	1.0±0.44 (33.3)	0.83±0.30 (33.3)	0.67±0.42 (21.2)
Snail Kill	1.67±0.21 (45.5) +	1.5±0.34 (42.8)	1.33±0.49 (39.9)	1.16±0.40 (36.7)	0.67±0.21 (40.2)
Pestoban	2.5±0.42 (57.7) +	1.5±0.34 (33.3)	1.33±0.5 (39.9)	1.33±0.42 (30.7)	0.83±0.40 (31.2)
Control (Agar)	4.33±0.21 (76.36)	3.83±0.16 (72.47)	4.5±0.34 (81.81)	4.16±0.16 (71.23)	3.5±0.34 (74.84)

Values in parentheses are percentages of snails in zone 3 (in contact with attractant food pellet) compared with snails in zone 1 and 2.

Statistically significant ($p < 0.05$) when two way ANOVA was applied in between different molluscicides (+) and their different concentrations (*).

Abbreviations: SP- Seed powder, BP- Bulb powder, BaP- Bark powder

69 cooling, the pellets were cut out from the layer with a
70 corer (5 mm diameter).

71 Assay and Apparatus

72 The bioassay was performed as reported earlier [14,
73 16]. The bioassay chamber consists of a clean glass
74 aquarium having a diameter of 30 cm. Each aquarium
75 was divided into four concentric zones; Zone 3 (central
76 zone), zones 2 and 1 (middle zone) and zone 0 (outer
77 zone) had diameters of 13, 18, 24 and 30 cm, respec-
78 tively. A small annular elevation of 9 mm height and 2.4
79 cm in diameter was made in the centre of aquarium
80 (Zone 3). Zone 0 had an area of 254 cm² on the periph-
81 ery of aquarium. The aquaria were then filled with 500
82 ml of dechlorinated tap water to a height of 8 mm and
83 maintained at 25±1⁰ C. At the start of the assay ten indi-
84 vidually marked snails of uniform size were placed on
85 the circumference of zone 0. The distance between two
86 snails was 66 mm. Simultaneously, one of the prepared
87 bait of different molluscicides was added on the small
88 annular elevation in the centre (Zone 3). The location of

89 each snail was recorded every 15 min for two hours. Six
90 sets of experiments were carried out with ten snails each
91 for every molluscicide used in this study. The mortality
92 of the snails was observed after the test with every 24h
93 up to 144h. Lethal values (LC₅₀), lower and upper con-
94 fidence limits (LCL and UCL), slope values, t- ratio, 'g'
95 value and heterogeneity factor were calculated using
96 POLO computer programme [17]. One/two-way
97 ANOVA and product moment correlation coefficient
98 was applied between the different data obtained in Ta-
99 bles 1-4 [18]. These experiments were repeated in stored
100 pellets kept for 7, 14, 21 and 28 days under laboratory
101 conditions.

102 RESULTS

103 Low attraction (45.5%) of the snails was observed
104 by the Snail Kill compared to plant derived mollus-
105 cides in zone 3 at 0.1% concentration in AFP (Table
106 1). 0.1% AFP containing *A. squamosa* seed powder, *A.*
107 *sativum*, *P. tuberosa* bulb powder, *A. indica* bark pow-

Table 2. Mean number of snail *L. acuminata* in zone three in contact with the stored attractant food pellets (AFP) containing 0.1% molluscicides.

Molluscicides	TIME OF STORAGE (IN DAYS)				
	0	7	14	21	28
<i>Pestoban</i>	2.5±0.42 (57.7) +	2.16±0.16 (50.0)	1.83±0.16 (61.1)	1.75±0.19 (43.2)	1.16±0.16 (41.2)
<i>Snail Kill</i>	1.67±0.21 (45.5) +	1.67±0.21 (44.2)	1.33±0.21 (36.3)	0.83±0.16 (38.2)	0.67±0.21 (34.5)
<i>A. indica</i> (Ba P)	3.5±0.34 (60.0) +	3.33±0.21 (55.5)	1.83±0.16 (47.7)	1.5±0.22 (40.9)	1.33±0.21 (34.5)
<i>A. sativum</i> (BP)	3.67±0.2 (78.0) +	4.0±0.36 (44.4)	3.16±0.47 (41.2)	2.72±0.51 (40.4)	2.16±0.16 (35.0)
<i>P. tuberosa</i> (BP)	3.0±0.25 (60.0) +	1.83±0.16 (31.3)	1.67±0.21 (35.7)	1.16±0.16 (27.8)	0.83±0.16 (31.2)
<i>A. squamosa</i> (SP)	1.16±0.16 (58.0) +	1.67±0.21 (35.7)	1.33±0.42 (30.7)	1.16±0.16 (27.8)	0.83±0.16 (31.2)
Acetogenin	3.0±0.36 (50.0) +	2.67±0.21 (47.0)	2.0±0.44 (40.0)	1.5±0.34 (33.3)	1.16±0.16 (27.8)
Azadirachtin	1.16±0.16 (46.4) +	0.83±0.16 (31.2)	0.83±0.16 (31.2)	0.67±0.42 (28.6)	0.5±0.22 (25.0)
Allicin	1.4±0.35 (46.7) +	1.33±0.42 (36.3)	1.16±0.16 (34.9)	0.83±0.3 (31.2)	0.83±0.3 (31.2)
Hecogenin	0.67±0.21 (50.3) +	0.67±0.21 (28.6)	0.67±0.21 (28.6)	0.5±0.22 (25.0)	0.33±0.21 (24.0)
Control (Agar)	4.5±0.34 (81.81)	4.66±0.21 (78.23)	5.5±0.16 (74.87)	4.33±0.21 (78.56)	5.33±0.47 (77.68)

Values in parentheses are percentages of snails in zone 3 (in contact with the stored attractant food pellet) compared with snails in zone 1 and 2.

+ Statistically significant ($p < 0.05$) when one way ANOVA was applied in between the number of snails in different storage period of bait formulations.

Abbreviations as in table 1.

Table 3. Toxicity in different bait formulations of molluscicides against the snail *L. acuminata* at different time exposure.

Expo-sure Period	Molluscicides	LC ₅₀ % in AFP	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h	<i>A. sativum</i>	1.57	1.07	4.38	1.80±0.45	3.92	0.24	0.28
	<i>P. tuberosa</i>	2.07	1.5	5.0	2.09±0.54	3.87	0.25	0.17
	Allicin	1.35	0.84	4.1	1.45±0.33	4.38	0.20	0.32
	Hecogenin	1.54	1.04	6.0	1.9±0.57	3.34	0.34	0.13
	Snail Kill	1.36	1.04	2.56	2.52±0.57	4.38	0.19	0.30
48h	<i>A. sativum</i>	1.53	0.93	11.29	1.12±0.37	3.03	0.41	0.15
	<i>P. tuberosa</i>	1.19	1.00	1.60	2.41±0.49	4.84	0.16	0.15
	Allicin	0.92	0.59	2.59	1.14±0.27	4.15	0.22	0.16
	Hecogenin	0.90	0.74	1.32	2.34±0.51	4.52	0.19	0.16
	Snail Kill	1.30	0.97	2.68	2.07±0.50	4.08	0.23	0.22
72h	<i>A. sativum</i>	0.86	0.61	2.0	1.16±0.34	3.35	0.34	0.22
	<i>P. tuberosa</i>	1.01	0.85	1.33	2.12±0.48	4.36	0.20	0.27
	Allicin	0.32	0.24	0.45	1.34±0.25	5.21	0.14	0.28
	Hecogenin	0.70	0.58	0.93	2.0±0.48	4.33	0.20	0.15
	Snail Kill	0.94	0.76	1.43	2.08±0.46	4.48	0.19	0.16
96h	<i>A. sativum</i>	0.44	0.28	0.61	1.19±0.33	3.55	0.30	0.21
	<i>P. tuberosa</i>	0.76	0.64	0.88	2.61±0.50	5.22	0.14	0.29
	Allicin	0.20	0.14	0.27	1.36±0.25	5.30	0.13	0.21
	Hecogenin	0.55	0.45	0.67	2.15±0.47	4.53	0.18	0.20
	Snail Kill	0.68	0.55	0.91	1.84±0.44	4.19	0.21	0.16
120h	<i>A. sativum</i>	0.28	0.08	0.42	0.99±0.33	2.99	0.42	0.28
	<i>P. tuberosa</i>	0.57	0.46	0.66	3.26±0.56	5.80	0.11	0.36
	Allicin	0.12	0.07	0.15	1.68±0.28	5.99	0.10	0.20
	Hecogenin	0.39	0.27	0.46	2.11±0.47	4.43	0.19	0.20
	Snail Kill	0.41	0.29	0.51	1.89±0.44	4.28	0.20	0.18
144h	<i>A. sativum</i>	0.18	0.07	0.26	1.46±0.35	4.15	0.22	0.34
	<i>P. tuberosa</i>	0.48	0.38	0.55	3.92±0.70	5.55	0.12	0.44
	Allicin	0.09	0.05	0.12	2.03±0.33	6.11	0.10	0.42
	Hecogenin	0.27	0.17	0.34	2.63±0.53	4.95	0.15	0.53
	Snail Kill	0.33	0.25	0.40	2.90±0.50	5.82	0.11	0.31

Product moment correlation showed significant ($p<0.05$); negative correlation in between the exposure period and LC₅₀ of different molluscicides.

Abbreviations as in table 1.

108der, their active components i.e. acetogenin, allicin, 1373 and 4). The crude preparations of plant derived mol-
109hecogenin, azadirachtin and pestoban caused more at-138luscicides and AFP containing pestoban caused signifi-
110traction (58.0%, 78.0%, 60.0%, 60.0% 50.0%, 46.7%, 139cant molluscicidal activity against *L. acuminata* (Table
11150.3%, 46.4% and 57.7%, respectively) than the AFP1403 and 4).

112containing 0.2% to 1.0% of the same molluscicides. The141 The slope values given in Tables 3 and 4 were steep.
113attraction of the snails was significantly ($p<0.05$) re-142Separate estimate of LC₅₀ based on each of the six repli-
114duced with increasing concentration of different mollus-143cates was found to be within 95% confidence limits.
115cicides in AFP. Lowest attraction (13.2%) of snails was144The t- ratio was greater than 1.96 and the heterogeneity
116observed for 1.0% hecogenin containing AFP. There145less than 1.0. The 'g' value was less than 0.5 at all prob-
117was a significant ($p<0.05$) decrease in the number of the146ability levels (90, 95, 99).

118snails attracted by all other 0.1% stored AFP for 7, 14,

11921 and 28 days except *A. sativum* containing AFP (Ta-

120ble2).

121 Molluscicidal activity of different AFP containing148 Higher attraction of the snails towards AFP contain-
122products/compounds against *L. acuminata* followed a149ing 0.1% plant derived molluscicides compared with
123time and dose dependence relationship (Table 3-4).150Snail Kill, appears to be due to the slower release of
124There was a significantly ($p<0.05$) negative correlation151molluscicidal compounds in comparison with synthetic
125between exposure period and LC₅₀ in different mollus-152ones. Higher concentration of plant derived mollus-
126cicides. AFP containing bioactive components of differ-153cicides and their active components in AFP caused less
127ent plants were more toxic (acetogenin 24h LC₅₀-1.02%154attraction than corresponding concentration of Snail
128in AFP; 144h LC₅₀-0.12% in AFP) than synthetic ones.155Kill. It indicates that when higher titer of active compo-
129The molluscicidal activity of garlic crude bulb powder156nents of plant derived molluscicides was used in AFP,
130(144h LC₅₀-0.18% in AFP) was higher than Snail Kill157snails were less attracted. Higher concentration (1.0%)
131(144h LC₅₀-0.33% in AFP). Allicin was more toxic158of allicin, hecogenin and azadirachtin [19-21] attracted
132(144h LC₅₀-0.09% in AFP) than the crude bulb powder159less snails than Snail Kill. There was a significant de-
133of *A. sativum* (144h LC₅₀-0.18% in AFP). The bioactive160crease in the attraction of *L. acuminata* towards AFP
134components hecogenin, acetogenin and azadirachtin161containing molluscicides compared with AFP alone
135were more toxic (24h LC₅₀- 1.54%, 1.35% and 1.10% in162with a significant variation in mean number of snails in
136AFP, respectively) than their crude preparations (Table163zone 3 containing different concentrations of mollus-

DISCUSSION

Table 4. Toxicity in different bait formulations of molluscicides against the snail *L. acuminata* at different time exposure.

Exposure Period	Molluscicides	LC ₅₀ % in AFP	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h	<i>A. indica</i> BaP	1.48	0.86	6.98	1.24±0.33	3.76	0.27	0.18
	<i>A. squamosa</i>	1.53	1.23	2.57	3.18±0.74	4.27	0.21	0.26
	Azadirachtin	1.10	0.86	1.99	2.26±0.54	4.15	0.22	0.12
	Acetogenin	1.02	0.72	2.16	1.68±0.36	4.62	0.18	0.29
	Pestoban	2.82	2.24	5.71	3.14±0.86	3.62	0.29	0.23
48h	<i>A. indica</i> BaP	1.01	0.62	3.90	1.03±0.28	3.61	0.29	0.17
	<i>A. squamosa</i>	1.25	1.08	1.88	2.75±0.65	4.24	0.21	0.14
	Azadirachtin	1.03	0.78	2.14	1.82±0.50	3.64	0.28	0.13
	Acetogenin	0.78	0.54	1.75	1.23±0.29	4.22	0.21	0.19
	Pestoban	2.46	1.98	4.94	2.43±0.71	3.40	0.33	0.23
72h	<i>A. indica</i> BaP	0.53	0.37	1.09	1.05±0.27	3.88	0.25	0.15
	<i>A. squamosa</i>	0.98	0.83	1.32	2.38±0.60	3.95	0.24	0.11
	Azadirachtin	0.73	0.59	1.11	1.78±0.47	3.75	0.27	0.13
	Acetogenin	0.39	0.29	0.58	1.23±0.27	4.57	0.18	0.21
	Pestoban	1.73	1.48	2.27	2.44±0.66	4.14	0.28	0.21
96h	<i>A. indica</i> BaP	0.25	0.17	0.34	1.23±0.26	4.66	0.17	0.22
	<i>A. squamosa</i>	0.63	0.50	0.73	2.71±0.61	4.45	0.19	0.11
	Azadirachtin	0.41	0.25	0.52	1.60±0.46	3.45	0.32	0.14
	Acetogenin	0.32	0.24	0.42	1.47±0.27	5.42	0.13	0.52
	Pestoban	1.33	0.99	1.60	2.07±0.63	3.26	0.36	0.28
120h	<i>A. indica</i> BaP	0.15	0.70	0.23	1.10±0.26	4.20	0.21	0.23
	<i>A. squamosa</i>	0.53	0.45	0.59	4.75±0.74	6.37	0.09	0.37
	Azadirachtin	-	-	-	-	-	-	-
	Acetogenin	0.19	0.10	0.27	1.09±0.26	4.18	0.22	0.49
	Pestoban	1.06	0.75	1.25	2.58±0.64	4.00	0.24	0.28
144h	<i>A. indica</i> BaP	0.09	0.03	0.14	1.27±0.27	4.57	0.84	0.36
	<i>A. squamosa</i>	0.48	0.40	0.54	5.32±0.88	6.04	0.10	0.37
	Azadirachtin	-	-	-	-	-	-	-
	Acetogenin	0.12	0.06	0.16	1.40±0.27	5.10	0.14	0.55
	Pestoban	0.96	0.75	1.10	3.65±0.68	5.32	0.13	0.86

Product moment correlation showed significant ($p < 0.05$); negative correlation between the exposure period and LC₅₀ of different molluscicides.

Abbreviations as in table 1.

cicides after two hours of exposure. AFP containing from the seeds of *A. squamosa* is higher than other plant acetogenin attracted more snails at lower concentrations derived molluscicides and Snail Kill (24h LC₅₀- 1.36% than *A. squamosa* seed powder. It indicates that *A.* in AFP). Seeds of *A. squamosa* were used to kill human *squamosa* seed powder, instead of acetogenin contains lice [22] and their organic extracts have been reported to some other compounds which reduce the attraction of possess insecticidal activity [23-24]. Molluscicidal ac-snails towards AFP. In contrast AFP containing *A. sati*-tivity of *A. indica* bark powder (24h LC₅₀ 1.48% in *vum* and *P. tuberosa* bulb powder and *A. indica* bark AFP) is lower than the Snail Kill. However, its active powder attracted more snails than their pure compounds component azadirachtin (24h LC₅₀ 1.10% in AFP) is viz. allicin, hecogenin and azadirachtin. It seems that more toxic than Snail Kill. Toxicity of AFP containing these plant derived molluscicides either contain some azadirachtin was effective only up to 96h. It indicates other compounds which attract the snails or the concen- that it is less stable in water or it is metabolized in snail trations of active molluscicidal components are less in body [20]. AFP containing hecogenin in AFP is 1.5 time crude preparations. The storage of attractant food pellets more toxic than the crude bulb powder of *P. tuberosa*. It for up to 28 days caused significant decrease in the at- has been reported that treatment of *P. tuberosa* bulb traction of snails. Thus, it seems logical to assume that powder and hecogenin caused significant reduction in AFPs containing plant derived molluscicides are less the reproduction of the *L. acuminata* [20]. It has been effective in attracting snails, when stored up to 28 days. reported that the allicin caused an uncompetitive inhibi- However, toxicity of these AFP containing mollus- sion of acetylcholinesterase and competitive inhibition cicides was time and dose dependent as evident from 209 of lactic dehydrogenase and alkaline phosphatase activ- the negative correlation between LC₅₀ in different mol- tivity in the nervous tissue of *L. acuminata* [25]. The toxic- luscicides and exposure period.

211ity of molluscicide Pestoban is due to the presence of Treatment of bulb powder of *A. sativum* and *P. tube*- *Cedrus deodara*, *A. indica* and *Embelia ribes* in liquid *rosa*, seed powder of *A. squamosa*, bark powder of *A.* concentrate form [26]. The toxicity of Pestoban to the *indica* and their active components such as allicin, snail is lower (24h LC₅₀ 2.82% in AFP) than the syn- hecogenin, acetogenin and azadirachtin in aquatic envi- thetic molluscicide Snail Kill (24h LC₅₀ 1.36% in AFP). ronment are highly toxic to *L. acuminata* [19-21]. Tox- The steep slope values indicate that a small increase 217 in the concentration in different molluscicides cause a

218 significant mortality in the snail. t- ratio value greater 267 11.
 219 than 1.96 indicates that the regression is significant. 268
 220 Values of heterogeneity less than 1.0 denote that in the 269
 221 replicates the concentration response line would fall 270 12.
 222 within 95% confidence limit and thus the model fit the 271
 223 data adequately. The value of 'g' is less than 0.5 indi- 272
 224 cates the index of significance of potency estimation. 273
 225 Use of plant derived molluscicides in aquatic envi- 274 13.
 226 ronments requires large amounts of molluscicides for 275
 227 effective control of snails. Using attractant food pellets 276
 228 like this study will be beneficial since it requires small 277 14.
 229 quantities of molluscicides while killing the target pest 278
 230 specifically. The present study shows that the use of 279
 231 AFPs containing plant derived molluscicides is very 280 15.
 232 effective in killing the snail *L. acuminata*. Use of these 281
 233 plant derived molluscicides inside the baits (Attractant 282
 234 food pellets) are ecologically sound, target specific and 283 16.
 235 economic. 284
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