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Inactivation of *Ascaris suum* by Short-Chain Fatty Acids[▽]

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***Ascaris suum* eggs were inactivated in distilled water and digested sludge by butanoic, pentanoic, and hexanoic acids. The fatty acids (short-chain fatty acids [SCFA]) were effective only when protonated and at sufficient concentrations. The conjugate bases were not effective at the concentrations evaluated. Predictions from an inhibition model (50% inhibitory concentration [IC_{50}]) based on quantitative structure-activity relationships were congruent with inactivation data.**

The nematode *Ascaris lumbricoides* releases highly resistant, unembryonated eggs into the environment, causing ~1.3 billion illnesses worldwide (12). The swine parasite *Ascaris suum* is routinely used as a surrogate for the human parasite (22) and is often found in sludges. Due to its resistance to biocontrol mechanisms (6) *Ascaris* is a model organism for developing environmentally safe disinfection methods (7, 22). The eggs can be rendered nonviable through natural processes using extreme heat (>40°C) or with UV radiation (4). The use of short-chain fatty acids (SCFA) is another possible method of controlling *Ascaris.* The toxicity of SCFA to bacteria, e.g., *Escherichia coli* (9), *Streptococcus*, and *Staphylococcus* (20), fungi (14, 23), insects (15), and birds (13) has been reported. Izumi (17) reported that acetic acid had limited ability to

TABLE 1. Percent viability of *Ascaris* eggs after exposure to fatty acids for 20 h

Acid(s)	pK_a	Molarity	Percent viability ± SD (pH) of *Ascaris* eggs at pH range[a]			
			2.0–4.0	4.0–4.5	4.5–5.0	5.0–6.0
Butanoic	4.76	0.1	91.6 ± 2.5 (2.0)	88.4 ± 1.7 a (4.0)	90.8 ± 1.1 (4.5)	91.7 ± 1.3 (5.0)
		1	0.0 ± 0.0 a (2.0)	0.0 ± 0.1 a (4.0)	0.1 ± 0.1 a (4.5)	1.2 ± 0.3 a (5.0)
		1.5	0.0 ± 0.0 a (2.0)	0.0 ± 0.0 a (4.0)	0.1 ± 0.1 a (4.5)	0.0 ± 0.1 a (5.0)
Pentanoic	4.78	6.3E-06	91.8 ± 1.6 (3.5)	89.9 ± 1.1	91.9 ± 0.2 (4.5)	93.6 ± 1.2 (5.0)
		0.01	88.4 ± 2.1 a (2.9)	89.9 ± 1.7 (4.0)	91.0 ± 2.6 (4.5)	88.6 ± 2.2 (5.0)
		0.1	86.4 ± 1.0 a (2.7)	92.7 ± 1.8 (4.0)	90.1 ± 1.0 (4.5)	87.1 ± 3.0 a (5.0)
		0.2	0.3 ± 0.3 (2.5)	0.2 ± 0.1 (4.0)	90.5 ± 1.4 (4.5)	90.8 ± 0.5 (5.0)
Hexanoic	4.85	6.6E-06	ND[b]	ND	ND	89.3 ± 0.5 (5.4)
		0.0001	ND	91.2 ± 0.7 (4.4)	88.2 ± 1.4 (4.5)	89.3 ± 0.5 (5.1)
		0.001	85.9 ± 1.0 (3.4)	91.1 ± 1.2 (4.0)	89.2 ± 2.0(4.5)	90.8 ± 1.8 a (5.0)
		0.0378	0.1 ± 0.2 a (3.1)	0.3 ± 0.1 a (4.0)	5.3 ± 0.7 a (4.5)	91.9 ± 1.9 a (5.0)
Butanoic and pentanoic[c]	4.76/4.78[d]	1.36/0.2[d]	ND	0.0033 ± 0.0058 b (4.0)	ND	90.0 ± 3.6 (6.0)
		0.136/0.2[d]	ND	1.98 ± 0.24 b (4.0)	ND	ND
		1.36/0.02[d]	ND	0.0033 ± 0.0058 b (4.0)	ND	ND
		0.136/0.02[d]	ND	91.38 ± 2.2 (4.0)	ND	ND

[a] Letters after viability measurements denote statistically significant differences among the percentages of viable eggs when compared to the water controls which were conducted with each round of testing (data not shown). The overall average percent survival for the water controls was 90.3 ± 2.7%. Data marked "a" were tested with the Proc Mix of SAS ($P < 0.01$). Data marked "b" were tested with the Student *t* test ($P < 0.05$). SD, standard deviation.
[b] ND, not done.
[c] The concentrations tested were chosen to reflect high concentrations and low concentrations.
[d] Values refer to butanoic acid and pentanoic acid, respectively.

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TABLE 2. Percent viability of *Ascaris* eggs after exposure to fatty acids in sludge and incubation at 37°C

Acid or sludge type	Molarity	% of *Ascaris* eggs viable[a] (±SD) after:	
		0.83 days	7 days
Propanoic	1.5	39.7 ± 3.1 a	0 ± 0 b
Butanoic	1.35	0 ± 0 a	0 ± 0 b
Pentanoic	0.2	0 ± 0 a	0 ± 0 b
Sludge control	None	92.3 ± 1.7	83.8 ± 0.4
Sludge, pH 4	None	93.3 ± 1.8	82.8 ± 7.2

[a] Letters a and b denote statistically significant differences among the percentage of viable eggs when compared to the sludge control at pH 4 as tested via the Student *t* test ($P < 0.05$). SD, standard deviation.

permeate the shells of *Ascaris* eggs. SCFA have also been used as an additive to disinfect silage or food and have been used to sanitize animal carcasses by spraying or dipping (8). In some cases, the action of the SCFA has been attributed to diffusion across the membrane, disassociation, and acidification of the cell interior (1, 3, 11). The work reported herein presents an evaluation of the efficacy of SCFA type, concentration, and pH on inactivation of *Ascaris* eggs in water and sludge. A model was developed that can predict the toxicity of SCFA-based concentration and pH.

Unembryonated *A. suum* eggs were collected from the intestinal contents of naturally infected pigs and cleaned (10). One thousand eggs were mixed with SCFA (the SCFA stock solutions were at their maximum water solubility without the use of a cosolvent) to a final volume of 1.0 ml. The reaction mixture was statically incubated at 37°C for 20 h. The eggs were collected by centrifugation, washed three times with phosphate buffer (pH 7.0, 10 mM), mixed with 0.5% Formalin solution and water, placed in a 24-well culture plate, and incubated at 28°C for 20 or 21 days. The outer coating was removed with 0.6% hypochlorite solution, and the eggs were scored as larvated (viable) or nonlarvated (not viable). Unless

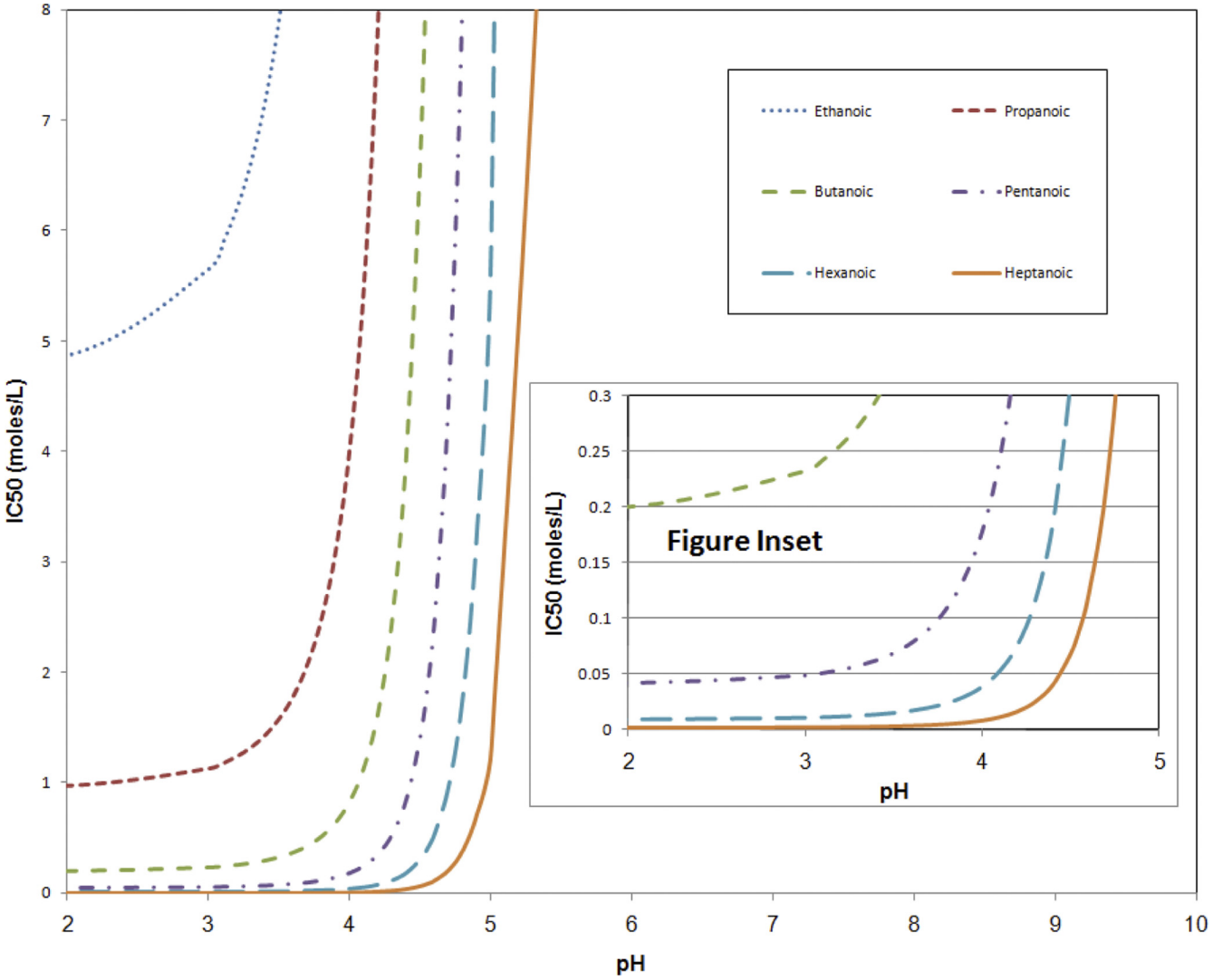

FIG. 1. Model of *Ascaris suum* inhibition (IC_{50}, moles/liter) as a function of pH. Model predictions were based on a quantitative structure-activity model (QSAR) according to a relationship reported by Kamlet et al. (18).

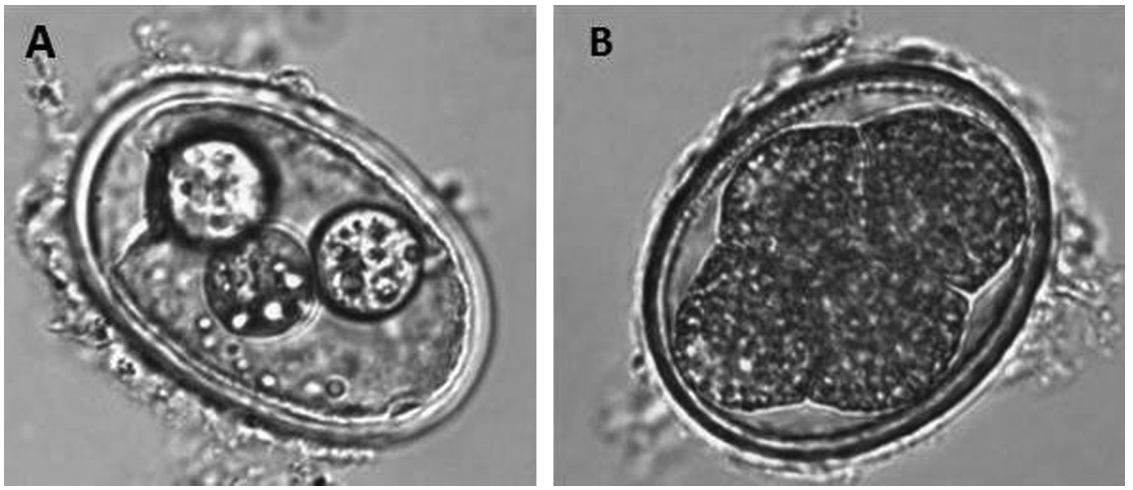

FIG. 2. (A) An *Ascaris suum* egg exposed to SCFA and exhibiting morphological damage to the interior cells. (B) A healthy *A. suum* egg.

otherwise stated, triplets were conducted. *A. suum* eggs (40,000) were mixed with untreated sludge collected from the final stage (prior to dewatering) of an anaerobic digester (Syracuse, NY) at pH 4 (100-ml final volume) and sludge amended with butanoic (1.35 M), propanoic (1.5 M), or pentanoic (0.2 M) acid. The sludge samples were incubated at 37°C for 20 h, 1 week, and 1 month. After the prescribed exposure period, 20 ml of sludge was removed and eggs were separated from the sludge by magnesium sulfate (specific gravity, 1.2) flotation and then washed and incubated at 28°C for 14 days as described above. After incubation, the eggs were decorticated and scored for viability. Unless otherwise stated, all experiments were conducted in triplicate.

Butanoic, pentanoic, and hexanoic acids all reduced the viability of the *Ascaris* eggs to below detectable limits (Table 1). The effect was most pronounced at pH values below the pK_a of the SCFA, and none of the SCFA were effective at pH 7 (data not shown). Mixtures of butanoic and pentanoic acids were also effective only at a low pH and at relatively high concentrations (Table 1). In the presence of sludge the SCFA were still effective at inactivating *Ascaris* eggs (Table 2). However, in this case propanoic acid exhibited toxicity at 20 h and completely inactivated the eggs after 7 days. Acetic acid was tested at 0.1, 0.5, 1.0, and 1.5 M and pH 2, 4, 4.5, 5, 6, and 7, while propanoic acid was tested at 1.5 M and pH 4 and 7. Neither acid appeared to be capable of reducing the viability below the control levels (data not shown). Heptanoic acid (6.3^{-6}, 1.0^{-5}, 1.0^{-4}, and 1.9^{-3} M and pH 3.7, 5.1, 6.1, 6.5, and 7.2), octanoic acid (4.72^{-4} M, pH 4.2), and nonanoic acid (1.0^{-4} M, pH 4.4) also did not appear capable of inactivating the eggs (data not shown).

The toxicity of the SCFA is dependent on several factors, including carbon chain length, SCFA concentration, pH, and K_{ow} (hydrophobicity). These factors, to determine nonreactive toxicity, were modeled with quantitative structure-activity relationships (QSAR). Nonreactive toxicity is related to the quantity of toxicant acting upon an organism and not directly associated with a specific mechanism (2). Blum and Speece (2) reported on numerous studies that successfully related aquatic toxicology on a surrogate organism to the logarithm of the octanol-water partitioning coefficient ($\log K_{ow}$). The inhibition of the acids on *Ascaris* was modeled using a surrogate organism (*Photobacterium phosphoreum*) according to the relationship reported by Kamlet et al. (18) as follows:

$$\log IC_{50} = 5.19 - 0.99(\log K_{ow})$$

The 50% inhibitory concentration (IC_{50}) is defined as the concentration of SCFA required to inactivate 50% of the *Ascaris* eggs. The values of K_{ow} were determined according to the method of Lyman (19), and changes in K_{ow} as a function of pH were determined as follows (16):

$$K_{ow}^{pH} = K_{ow,\,HA} f_{\alpha} + K_{ow,\,A} f_{1-\alpha}$$

where f_{α} and $f_{1-\alpha}$ are the fractions of neutral and ionized SCFA species, respectively, in aqueous solution. Because a surrogate organism was used, *Ascaris* inactivation data were not required to calibrate the model, a significant advantage of the QSAR model (2).

Figure 1 presents the required SCFA concentration to achieve inactivation of 50% (IC_{50}) for *Ascaris* as a function of pH. The model predicts a clear concentration barrier that must be reached in order for the SCFA to be toxic, and that concentration increases substantially at pH values slightly above the pK_a. The inactivation data in the tables and data not shown (e.g., octanoic acid) are congruent with the model predictions at pH values less than 4.5: all SCFA concentrations that were above and to the left of the model predictions resulted in a statistically significant inactivation greater than the IC_{50} of the *Ascaris* eggs, and concentrations below and to the right resulted in inactivation less than the IC_{50}. Blum and Speece (2) reported that a reasonable goal for the accuracy of a QSAR in predicting toxicity to bacteria is approximately one order of magnitude (concentration), based on the variability of toxicity tests and applying those tests to a field application. The performance of the model is consistent with this goal at a pH less than 4.5. The model predictions are also consistent with inactivation data for pH values above 4.5 with the exception of butanoic acid. In addition, mixtures of butanoic and pentanoic acids were not effective until they passed the threshold con-

centration (Fig. 1 and Table 1). These findings are consistent with those of Paggi and Fay (21), where acetic acid was less effective than propanoic and butanoic acids against *Streptococcus bovis*. The authors speculated that it was due to the higher pK_a and lower hydrophobicity of the former. The greater effect of the SCFA at lower pHs is likely due to the increased ability of the SCFA to permeate the membrane. The concentration of SCFA needed to decrease the growth by 50% decreased with increasing K_{ow}. The interaction between the protonated fatty acids and the eggs is likely hydrophobic, since the eggs (with and without the outer coat) are hydrophobic and lack electron acceptors (5). This explains the lack of effect by the deprotonated conjugate base. In addition, visual examination of some eggs showed that some of the internal components were disrupted, which indicates the SCFA may have altered the cell membranes (Fig. 2). This phenomenon was not quantified.

Based on the results of this work, selected SCFA were effective in the inactivation of *Ascaris* eggs in the protonated form and at sufficient concentrations. QSAR models are capable of predicating the inhibitory effects of SCFA. Butanoic and pentanoic acids can be added to sludge as a beneficial treatment to reduce the survival of *Ascaris* eggs prior to land application.

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