RESEARCH REPORT
SWINE HEALTH

Title: Determination of serum concentrations resulting from administration of high and low dose orally administered acetylsalicylic acid and sodium salicylate in swine through water medication systems – **NPB #04-034**

Investigator: Locke Karriker, DVM, MS

Institution: Iowa State University

Date Received: August 18, 2005

II. Abstract:

The objective of this study was to determine plasma concentrations in swine following water administration of a sodium salicylate liquid aspirin product. Initially, the solubility of an acetylsalicylic acid and sodium salicylate liquid aspirin product was tested by measuring active ingredient levels over twenty- four hours with high pressure liquid chromatography (HPLC) analysis. Significant differences in acetylsalicyclic acid and sodium salicylate product solubility was confirmed. The sodium salicylate product was used for the remainder of the trial.

Four sets of three pens of pigs (average weight of 18.19 ± 2.75 kg) in a commercial production nursery facility received one of four treatments (stock solution concentrations: T1 = 19.4ppm, T2 = 38.9ppm, T3 = 77.6ppm, T4 = 155.3ppm, and T5 = 0ppm) for a period of 72 hours. Blood samples were taken at 0, 24, 60, and 72 hours. Serum salicylate levels were measured using HPLC. Serum concentrations of sodium salicylate (measured by HPLC) for each treatment group are reported. This study indicates that sodium salicylate, when given orally through a water-medication system, is absorbed, and reaches measurable concentrations in the blood.

III. Introduction:

Aspirin is widely used in food animal production due to the current lack of antiviral drugs, the inexpensive cost, and the over-the-counter availability[2,3]. However, there is very little reported information on dosage and concentrations achieved in plasma. During a literature search, no dosage information could be found for aspirin administered via a water medication system. The dosage information that is reported in the USP Veterinary Pharmaceutical Information Monographs[17] and in Plumb's Drug Handbook[18] both cite work published by Davis[13]. While Davis's study gave important intravenous (IV) dosage information, this can not be used for water medication dosage as several factors, including oral bioavailability, are not included in the dosage calculations. Plasma concentrations of salicylate following administration of acetylsalicylic acid or sodium salicylate through swine water systems have not been reported.

Not only is oral dosage and plasma concentration information for aspirin scare, it is documented that aspirin has negative effects on the gastric mucosa in swine. In one study by Rainsford (1982), it was reported that aspirin, when given orally at a dose of 30 mg/kg/day and 200 mg/kg/day, significantly reduced mucosal and plasma prostaglandin concentrations[16]. The study also showed a correlation between mucosal damage and a reduction in mucosal prostaglandin. Interestingly, sodium salicylate has been shown to be less ulcerogenic then acetylsalicylic acid[22,23] and while it is a mild gastric irritant, it has a cytoprotective effect on gastric damage induced by selective cyclooxegenase-2 (COX-2) inhibitors[21,24].

Due to the widespread use of aspirin products, lack of oral dosage information, and potential harm to the animal, it was the aim of this study to determine plasma concentrations in swine following water administration of sodium salicylate.

IV. Objectives:

1. Measure and compare active ingredient levels in standard stock solutions of sodium salicylate and acetylsalicylic acid subjected to typical nursery barn conditions during a twenty-four hour period.
2. Selecting the most stable from phase 1, determine acetylsalicylic acid and sodium salicylate concentrations in the serum of pigs after administration through water medication systems.
3. To relate these concentrations to serum concentrations established as effective in other species and then use these data for dosage optimization in the design of a proposal for a follow up study evaluating the benefit of acetylsalicylic acid and sodium salicylate in the face of viral disease challenge.

V. Materials and Methods:

The pens from which pigs were to be sampled were plumbed with new pipes and equipment from the nipple to the fresh water source. No previous medication had been administered through the system at study initiation. One-half inch internal diameter PVC pipe, 5/8" Swan garden hose, Lixit 1/2" nipple waterers, and 1/2" galvanized pipes were used. A water sample was taken from the site and tested for coliforms, nitrates, sulfates, pH and iron.

A representative liquid sodium salicylate product and a representative liquid acetylsalicylic acid product from those commercially available were selected. The maximum solubility of each product in stock solution was determined by using the reported solubility.[12]

To confirm the solubility calculation, each product was used to formulate a stock solution with equivalent concentration of active ingredients (11.2 mg/ml). These stock solutions were placed in the test nursery environment for 24 hours and sampled at 0, 8, 16, and 24 hours. The concentration of salicylate was determined by a previously described High Pressure Liquid Chromatography (HPLC) procedure[10]. In addition, the experiment was repeated using a different water source. Appearance of the solutions was photographed and compared to the appearance of the stock solution from the nursery environment. Due to the higher solubility of the sodium salicylate product it was used for the remainder of the trial.

Four groups of three pens in a commercial production nursery facility were plumbed with group specific water medicators and water meters. Three other pens, comprising a fifth control group, were plumbed with a water meter only. Pens were populated and managed according to the standard operating procedures of the facility. The use of animals in this study was approved by the Committee on Animal Care at Iowa State University.

When pigs were approximately forty pounds average weight, each set of pens received one of the four treatments for a period of 72 hours. Stock solutions were prepared at the following concentrations: 0.3 oz/gal (19.4 ppm), 0.7 oz/gal (38.9 ppm), 1.3 oz/gal (77.6 ppm), 2.66 oz/gal (155.3 ppm), respectively for treatments 1-4. The first blood sample was collected from ten randomly selected pigs in each pen. These

pigs were tagged and additional samples were collected at 24, 60, and 72 hours after study initiation. Following the last bleeding, the pigs were weighed using an ElectroSampson digital scale. Hematocrit percentage was determined from the 72 hour blood samples. Serum salicylate levels were measured using HPLC based on a previously published protocol[10].

Statistical analysis was done using a MANOVA repeated measures test to determine whether there was a significant difference between treatment groups at all time points. Since a significant Wilke's Lambda was found, an ANOVA test was run using Dunnett's Method (with control group) to determine if there were significant differences between treatment groups at the various time points.

VI. Results:

The water sample from the nursery site (tested by a certified water testing facility) was found to have coliform bacteria at less then 1 per 100 ml total, a sulfate concentration of 78 mg/L, and less then 0.5 mg/L nitrate and nitrite. All three of these are well under the maximum contaminant level (MCL) allowed by the safe drinking water act as described in the report from the testing facility. The pH of the water was 6.305 as determined by measurement with an Omega digital pH meter. The level of copper and iron in the water were tested by the Iowa State University Veterinary Diagnostic Laboratory and found to be < 0.1 ppm and 0.6 ppm respectively.

Water consumption (assuming a 25% disappearance rate for the Lixit nipple[19]), mean pig weight (mean weight for all treatment groups was 18.19 ± 2.75 kg) and pig population for each treatment group are shown in Table 1. Statistical analysis of the hematocrit data showed no significant differences among the treatment groups.

Figure 1a, b, and c show scatter plots of serum concentrations at 24, 60, and 70 hours, respectively, with 95% confidence intervals. At the 24 and 72 hour time point, treatment 3 and 4 were significantly different ($p < 0.05$) then the control group. At the 60 hour time point, treatment 4 was significantly different from the control group. Table 2 shows the mean serum concentration (µg/mL) (standard deviation in parenthesis) of sodium salicylate by treatment and time. The peak serum concentrations were observed at 24 hours. Table 3 shows the concentration of sodium salicylate (mg/ml) in the stock solutions as determined by HPLC analysis for each time point. Average concentrations (mg/ml) over the 72 hour trial from HPLC measurements and the calculated average concentrations (mg/ml) are also given. Table 4 shows the concentrations of sodium salicylate in the water nipple samples (µg/ml) as determined by HPLC analysis for each time point. Average concentrations (µg/ml) over the 72 hour trial from HPLC measurements and the calculated average concentrations (µg/ml) are also given.

VII. Discussion:

Only liquid aspirin products were included in this study due to product availability, widespread use, and solubility. The liquid aspirin products currently available on the market were compiled and compared. At the time of the trial, three companies (herein labeled A-C) produced aspirin products. Company A produced both an acetylsalicylic acid and a salicylate product; company B only manufactured an acetylsalicylic acid product and company C only manufactured a salicylate product. All other products on the market were manufactured by company A and sold under various labels. All acetylsalicylic acid and salicylate liquid products claimed to have the same concentration of active ingredient, 120mg/ml and 485.6 mg/ml respectively. Therefore, company A's products were chosen for this trial.

Under both nursery and laboratory conditions the acetylsalicylic acid stock solution, prepared at a concentration of 11.2 mg/ml, crystallized out of solution. The experimental result that the acetylsalicylic acid product was less soluble then the sodium salicylate product agrees with documented solubility data;

specifically that the solubility of acetylsalicylic acid in water is 3.43 g/L while the solubility of sodium salicylate in water is 999.63g/L[12]. The difference seen in solubility also agree with the result that the sodium salicylate product retained more active ingredient then the acetylsalicylic acid product when analyzed by HPLC.

Given the above solubility data, the theoretical highest achievable daily dose of acetylsalicylic acid in swine is 3.1 mg/kg BW while the theoretical highest achievable daily dose of sodium salicylate in swine is 894.3 mg/kg BW. (These calculations assume a 40lb pig drinks an average of 0.55 gal per day and the medication is metered at a rate of 1:128.) Based on these solubility calculations, it seems likely that sodium salicylate products would be more effective should the therapeutic dose for aspirin be shown to be above 3.1mg/kg BW daily.

During the trial, water consumption among treatment groups was documented by recording water meter readings and hematocrit analysis was performed on blood samples. There was no indication that the pigs in a certain treatment group were more dehydrated then any other group (no significant difference between hematocrit data among all treatments).

The scatter plots of the serum concentrations at all time points confirm that sodium salicylate is absorbed when administered via a water medication system. The 24 hour plot also indicates that the serum concentrations are dose dependent. It is also evident that as the dose increased, the variance of the data also increased.

The serum concentration table shows an increase in concentration until 24 then a decrease in concentration in treatment group 4 (17.78 mg/kg BW dose) and treatment group 2 (4.45 mg/kg BW dose). The drop in serum concentration from the 24 to the 60 hour time period in treatment 4 is potentially due to a malfunction of the water medicator associated with this treatment group that occurred during the 24-48 hour time period. The malfunction of the water medicator was corrected prior to the 48 hour time point and functionality was verified. Therefore, the continuing drop in serum concentration seen in the 70 hour sample mean is not likely explained by a water medicator malfunction. Sample evaporation was also ruled out as a possible cause of the low serum concentrations as each stock solution was covered after preparation.

Control samples were taken from the water nipples and the stock solutions to ensure that the concentrations in the water and stock solution were accurate. Table 4 shows that the concentration in the water dropped considerably at the 60 hour time point for treatment groups 2, 3, and 4 and at the 72 hour time point for treatment groups 2 and 3. This correlates with the drop in serum concentration described above. The decline in the 60 hour time point may be partly due to the lower stock solution concentrations (table 3), but this explanation cannot fully account for the lower water nipple concentrations (shown in table 4). Due to differences between the theoretical and actual water concentrations of sodium salicylate, the experiment should be repeated to verify the results. This would also allow higher doses to be tested during the experiment.

Although co-variables, such as water quality, equipment types, and management styles (including vaccination and treatment protocols) must be taken into account when extrapolating this data to other production situations, the results of this trial can be used in future studies to develop dosage recommendations.

VIII. Lay interpretation

The first portion of this trial was designed to compare the solubility of two common liquid aspirin products – namely an acetylsalicylic acid and a sodium salicylate product. While the solubilities of these products are documented, the goal was to determine if under common commercial settings the solubility affected how much of the product would be taken up by the water medicator. Therefore, stock solutions were mixed at a

set concentration and placed under nursery conditions. The stock solutions were then sampled at four different time points. The samples were then analyzed to determine the amount of active ingredient present at each time point. The results indicated that the sodium salicylate product was more soluble, resulting in a higher concentration of active ingredient in the samples at all time points. Because the sodium salicylate product could deliver a higher concentration of active ingredient to the pigs it was used in the remainder of the trial. The fact that the sodium salicylate product can deliver more active ingredient doesn't necessarily imply that it should be used instead of acetylsalicylic acid products because the amount of aspirin that is beneficial is still unknown. If the amount of aspirin needed to reduce fever and pain enough to keep pigs on feed during a viral infection is relatively small, then either product would reach appropriate concentrations through the water medication system.

The second portion of the trial focused on administering aspirin at various stock solution concentrations and then measuring the resulting concentration of aspirin that was in the pig's blood. This is an important step in determining what dose of aspirin is beneficial, as information on drug absorption and distribution, two major factors in dosage determination, are unknown for this administration system in the pig.

The results indicate that the sodium salicylate product, when given orally through a water-medication system, is absorbed and reaches measurable concentrations in the blood.

Although co-variables, such as water quality, equipment types, and management styles (including vaccination and treatment protocols) must be taken into account when extrapolating this data to other production situations, the results of this trial can be used in future studies to develop dosage recommendations.

In conclusion, this study showed that (1) there are significant differences in the acetylsalicyclic acid and sodium salicylate product solubility which may lead to differences in dosage recommendations and (2) sodium salicylate, when given orally through a water-medication system, is absorbed and reaches measurable concentrations in the blood.

Table 1. Water intake and mean weight for each treatment group. T1 = stock solution concentration of 19.4 ppm, T2 = 38.9 ppm, T3 = 77.6 ppm, T4 = 155.3 ppm, T5 = 0.00 ppm.

	T1	T2	T3	T4	T5
Water disappearance (L/pig/day)	5.27	5.19	7.29	4.73	5.12
Water intake (L/pig/day) *	3.95	3.89	5.47	3.55	3.84
Water intake (gal/pig/day)*	1.04	1.03	1.45	0.94	1.01
Population (# of pigs)	51	49	40	51	46
Mean weight (kg)	18.72	16.98	17.77	17.79	19.73

*Assumes a wastage of 25%

Table 2. Mean serum concentration (µg/mL) (standard deviation in parenthesis) of sodium salicylate by treatment and time. T1 = stock solution concentration of 19.4 ppm, T2 = 38.9 ppm, T3 = 77.6 ppm, T4 = 155.3 ppm, T5 = 0 ppm.

Sample time (hrs)	T 1	T 2	T 3	T 4	T5
0	0	0	0	0	0
24	0.41(0.31)	1.28 (1.03)	1.41 (0.64)	7.22 (2.31)	0
60	0.17 (0.15)	0.82 (0.77)	0.44 (0.50)	2.66 (2.41)	0
72	0.27 (0.20)	0.03 (0.07)	1.24 (0.79)	0.62 (0.48)	0

Table 3. Concentrations of sodium salicylate in the stock solution (mg/ml) as determined by HPLC analysis for each time point. Average concentrations (mg/ml) over the 72 hour trial calculated from HPLC measurements and the theoretical average concentrations (mg/ml) are also given.

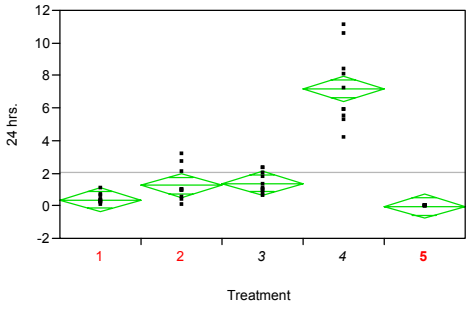
	Conc. in SS (mg/ml)			Actual Average (mg/ml)	Calculated Average (mg/ml)
Treatment	**24hr**	**60hr**	**72hr**		
1	2.4	1.35	2.35	2.03	2.48
2	5.1	4.2	4.95	4.75	4.97
3	10.3	7.15	10.35	9.27	9.94
4	18.1	13.75	17.4	16.42	19.87

Table 4. Concentrations of sodium salicylate in water nipple samples (µg/ml) as determined by HPLC analysis for each time point. Average concentrations (µg/ml) over the 72 hour trial calculated from HPLC measurements and the theoretical average concentrations (µg/ml) are also given.

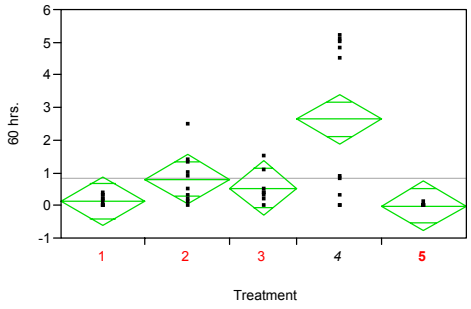
	Conc. in H_20 (µg/ml)			Actual Average (µg/ml)	Theoretical Average (µg/ml)
Treatment	**24hr**	**60hr**	**72hr**		
1	11.5	11	12	11.50	19.38
2	39	18	15.5	24.17	38.86
3	76.5	3.5	46	42.00	77.64
4	118.5	4.5	108.5	77.17	155.27
5	0	0	0	0	0

Figure 1a,b,c. One-way ANOVA analysis of serum concentrations (y-axis) by treatment groups (x- axis) at 24 hrs (1a), 60 hrs (1b), and 72 hrs (1c). T1 = stock solution concentration of 19.4 ppm, T2 = 38.9 ppm, T3 = 77.6 ppm, T4 = 155.3 ppm, T5 = 0 ppm.
Diamond peaks indicate 95% confidence intervals, middle line of diamond indicates the group mean. Black italic treatment numbers indicate groups that are significantly different ($p < 0.05$) from the control group (5) using Dunnett's method.

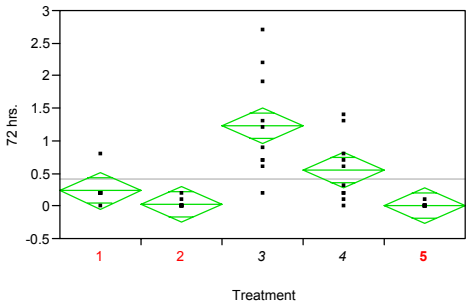
1a



1b



1c



References:

1. Davis, L.E. 1980. Clinical pharmacology of salicylates. *JAVMA*. 176:65-66.

2. Damian, P. 1997. Extra label use of nonsteroidal anti-inflammatory drugs. *JAVMA*. Oct;211(7):860-1.

3. Apley, M.D. 2004. Personal Communications.

4. Holvoet, J., L Terriere, W Van Hee, L Verbist, E Fierens, M L Hautekeete. Relation of upper gastrointestinal bleeding to non-steroidal anti-inflammatory drugs and aspirin: a case-control study. *Gut.* 1991;32(7):730-734.

5. Kun-Yu Wu, K. Biochemical Pharmacology of Nonsteroidal Anti-Inflammatory Drugs. *Biochem Pharm.* 1998;55:543-547.

6. Jenkins, W.L. Pharmacological aspects of analgesic drugs in animals: An overview. *J Am Vet Med Assoc.* 1987;191(10):1231-1240.

7. Vane, J. and Botting, R. Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am J Med*. 1998;104(3A):2S-8S; discussion 21S-22S.

8. Kopcha, M., et al. Use of nonsteroidal anti-inflammatory drugs in food animal practice. *J Am Vet Med Assoc*. 1992;201(12):1868-72.

9. Karriker 2003. Personal Communications.

10. Cham, B. E., et al. Measurement and pharmacokinetics of acetylsalicylic acid by a novel high performance liquid chromatographic assay. *Ther Drug Monit*. 1980;2(4):365-72.

11. Koritz, G.D., et al. Bioavailability of four slow-release theophyline formulations in the beagle dog. *J Vet Pharm Ther.* 1986;9(3):293-302.

12. Merck Co. *The Merck Veterinary Manual. 11th ed.* Rahway, NJ. Merck Company. 1999.

13. Davis, Lloyd E. and Bertis A. Westfall. Species differences in biotransformation and excretion of salicylate. *Am J Vet Res.* 1972;33(6):1253-1262.

14. Bochner, F., D.M. Siebert, S.E. Rodgers, G.H. McIntosh, M.J. James, and J.V. Lloyd. Measurement of aspirin concentrations in portal and systemic blood in pigs: effect on platelet aggregation, thromboxane and prostacyclin production. *Throm Haemos* 1989;61(2):211-216.

15. Eppler, Jeffrey, David Johnson, Zul Verjee, Esther Giesbrecht, Shinya Ito. Measurement of serum acetylsalicylic acid in a porcine model of aspirin overdose. *Vet Human Toxicol.* 1996;38(6):409-412.

16. Rainsford, K.D, and Carolyn Willis. Relationship of fastric mucosal damage induced in pigs by anti-inflammatory drugs to their effects on prostaglandin production. *Dig Dis Sci.* 1982;27(7):624-635.

17. USP Veterinary Pharmaceutical Information Monographs – Anti-inflammatories. *J Vet Pharmacol Ther. 2004;27(S1):1-13.*

18. Plumb, Donald C. Aspirin. *Veterinary drug handbook 4th ed. 2002.* Accessed online at <http://www.vin.com/Members/Drug/VDH.plx?ID=36>.

19. Yuzhi, Li and Harold. W. Gonyou. Water intake and wastage by grower/finisher pigs at nipple drinkers. *Prairie Swine Centre Inc. Publications.*

20. Rainsford, K.D. Gastric mucosal ulceration induced in pigs by tablets but not suspensions or solutions of aspirin. *J Pharm Pharmac.* 1978;30:129-130.

21. Amann, R. and B. A. Peskar. Anti-inflammatory effects of aspirin and sodium salicylate. *Eur J Pharmocol.* 2002;447:1-9.

22. Robert, Andre. Gastric cytoprotection by sodium salicylate. *Prostaglandins.* 1981;21 Suppl:139-46.

23. Whittle, B.J.R., G.A. Higgs, K.E. Eakins, S. Moncada, and J.R. Vane. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature.* Mar 20,1980;284:271-273.

24. Peskar, Brigitta M., Karlheinz Ehrlich, Bernhard A. Peskar. Interaction of cyclooxygenase-2 inhibitors and salicylate in gastric mucosal damage. *Eur J of _Pharmacol.* 2002;434:65-70.