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Dermal Toxicology Application Area Changes as Compared to a Theoretical Total Surface Area of Hanford Miniature Swine Over 18 Weeks

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ABSTRACT

Dermal toxicology studies are frequently initiated in growing animal models. The test compound is generally applied on a dermal application area (DAA) that is frequently set at certain ratio to total surface area. Since the growth during juvenile period can be significant, the DAA is likely to be impacted during the course of a chronic study. The purpose of this study was: (1) to determine if the ratio of the DAA to total body surface area (TBSA) will change proportionally over time with the growth of the miniature swine; and (2) to compare three TBSA formulas. The ratio DAA (cm^2 surface area) to TBSA was calculated from body weight at periodic intervals for 18 weeks in 16 male and 16 female Hanford miniature swine (~four-month-old Hanford miniature swine, averaging ~14 kg at initiation). Two midback 5 cm x 5 cm (25 cm^2) DAAs, one located on each side of the spine, were used on each subject. The TBSA (m^2) was calculated by the well-recognized Spector formula (9.5 x $BW(G)^{2/3}$/10000,) as well as the Brodie and Wachtel formulas. Using the Spector formula, the mean ratio DAA to TBSA for Weeks 0 and 8 (N = 32) for an application area was 0.46% ± 0.04 and 0.51% ± 0.06 (MEAN ± SD), respectively. After Week 8, subsequent periodic measurements of the ratio of TBSA to DAA remained steady, suggesting proportional changes in growth of both DAA and TBSA. The correlation of the three TBSA formulas was >0.99. Thus, comparable TBSA and DAA/TBSA ratio between the three formulas suggested the well-recognized Spector Method is a valid choice.

OVERALL RESEARCH OBJECTIVES

The purposes of this study were to:
1. Determine if the ratio of the DAA to TBSA changes proportionally over time in a young Hanford miniature swine model.
2. Compare the Spector TBSA estimate to Brodie and Wachtel formulas estimates.

HYPOTHESIS

1. The ratio of the DAA to TBSA changes proportionally as body weight increases.
2. The Spector TBSA formula will be comparable to other recognized formulas, namely the Brodie and Wachtel formulas.

STUDY METHODS

1. Determine the photobiological response of non-pigmented miniature swine skin (Hanford miniature swine).
2. Demonstrate minimal erythema dose (MED) for the Hanford strain of miniature swine.
3. Demonstrate dose-response for 8-MOP, an accepted positive control agent.
4. Evaluate selected skin biopsy specimens histopathologically.

STUDY ENDPOINTS

1. Calculate the TBSA (cm^2) by using swine factor formula 9.5 x $BW(G)^{2/3}$/10000 by Spector and the Brodie and Wachtel formulas.
2. Calculate the DAA (cm^2 surface area) by measuring and squaring the dimensions of the application area.
3. Calculate the DAA (cm^2 surface area) to TBSA ratio from body weight at periodic intervals over 18 weeks in 32 ~four-month-old Hanford miniature swine.

EXPERIMENTAL APPROACHES

1. The corners of the DAA were marked on either side of the animal at the midback area between shoulders and rump using permanent tattooing at the beginning of the study.
2. The DAA dimensions were 5 x 5 cm (25.0 cm^2) at initiation (Week 0). DAA were remeasured at 8, 12, and 18 weeks.
3. The coefficient factor of 9.5 (Spector, 1956) and the animal's body weight were used to calculate the TBSA with Spector's formula.
4. The ratios of DAA (cm^2 surface area) to TBSA were determined and the mean ratio by timepoints were compared using T-test (two tails).
5. A correlation analysis was performed between the TBSA estimated from the Spector formula and the Brodie and Wachtel formulas, respectively

EXPERIMENTAL RESULTS

Table 1. Measured DAA* as Percentage of Calculated TBSA** in 32 Young Adult Hanford Miniature Swine Over 18 Weeks: Spector Method

Week	Mean Body Weighttt (kg)	Mean TBSA (cm^2)	Mean TBSA (cm^2)	Mean Left DAA (cm^2)	Mean Right DAA as Percentage of TBSA	Right % Std Dev	Mean Left DAA as Percentage of TBSA***	Left % Std Dev
0	13.87	5473.2	25.0	25.0	0.46	0.04	0.46	0.04
8	20.45	7055.4	35.9	36.8	0.51	0.06	0.52	0.05
12	25.56	8239.1	40.4	43.0	0.49	0.05	0.52	0.04
18	34.60	10082.7	51.4	51.4	0.51	0.05	0.51	0.06

*DAA = Dermal Application Area; **TBSA= Total body surface area as calculated by Spector formula; ***Weeks 8, 12, and 18 were statistically different from Week 0 by two-tailed T-test at ≤ 0.01

Table 2. Measured DAA* as Percentage of Calculated TBSA** in 32 Young Adult Hanford Miniature Swine Over 18 Weeks: Brodie Method

Week	Mean Body Weight (kg)	Mean TBSA (cm^2)	Mean TBSA (cm^2)	Mean Left DAA (cm^2)	Mean Right DAA as Percentage of TBSA	Right % Std Dev	Mean Left DAA as Percentage of TBSA***	Left % Std Dev
0	13.87	5473.2	25.0	25.0	0.49	0.04	0.49	0.04
8	20.45	7055.4	35.9	36.8	0.55	0.06	0.57	0.05
12	25.56	8239.1	40.4	43.0	0.54	0.06	0.57	0.05
18	34.60	10082.7	51.4	51.4	0.56	0.05	0.56	0.06

*DAA = Dermal Application Area; **TBSA= Total body surface area as calculated by Brodie formula; ***Weeks 8, 12, and 18 were statistically different from Week 0 by two-tailed T-test at ≤ 0.01

Table 3. Measured DAA* as Percentage of Calculated TBSA** in 32 Young Adult Hanford Miniature Swine Over 18 Weeks: Wachtel Method

Week	Mean Body Weight (kg)	Mean TBSA (cm^2)	Mean TBSA (cm^2)	Mean Left DAA (cm^2)	Mean Right DAA as Percentage of TBSA	Right % Std Dev	Mean Left DAA as Percentage of TBSA***	Left % Std Dev
0	13.87	5473.2	25.0	25.0	0.46	0.03	0.46	0.03
8	20.45	7055.4	35.9	36.8	0.53	0.06	0.54	0.05
12	25.56	8239.1	40.4	43.0	0.52	0.05	0.55	0.04
18	34.60	10082.7	51.4	51.4	0.56	0.05	0.56	0.06

*DAA = Dermal Application Area; **TBSA= Total body surface area as calculated by Wachtel formula; ***Weeks 8, 12, and 18 were statistically different from Week 0 by two-tailed T-test at ≤ 0.01

Table 4. Comparison of Three Methods for Calculating TBSA

Week	Mean Body Weight (kg)	Mean Spector* TBSA (cm^2)	Mean Brodie** TBSA(cm^2)	Mean Wachtel*** TBSA (cm^2)	Mean Difference Spector vs. Brody	Mean Difference Spector vs. Wachtel
0	13.87	5473.2	5114.6	5477.1	-358.6	3.89
8	20.45	7055.4	6509.9	6820.3	-545.5	-235.1
12	25.56	8239.1	7542.8	7796.6	-696.3	-442.5
18	34.60	10082.7	9137.0	9279.8	-945.8	-802.9

Spector (1956) Surface Area cm^2 = 9.5 x $BW(G)^{2/3}$/10000 (Excel =9.5*POWER(W*1000,2/3)/10000*10000); **Brodie (1928) Surface Area cm^2 = 970*$W^{0.633}$ (Excel =POWER(W,0.633)*970); ***Wachtel (1972) Surface Area M^2 = 0.121*$W^{0.575}$ (Excel = POWER(W,0.575)*1210) for cm^2

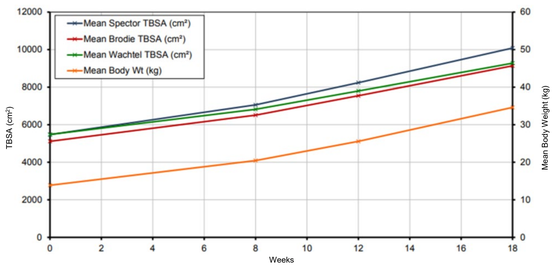

Figure 1. Mean TBSA by Spector, Brodie, and Wachtel Calculation Methods

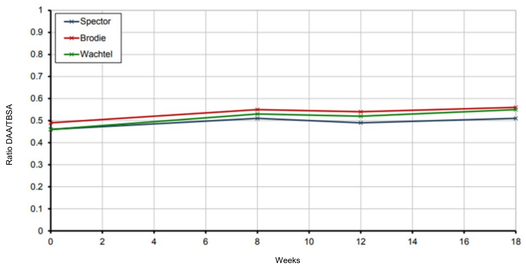

Figure 2. Mean Right DAA as a Percentage of Calculated TBSA

Table 5. Correlation of Three TBSA Calculation Methods

Method	Spector	Brodie	Wachtel
Spector	1.0	0.9999	0.9999
Brodie	0.9999	1.0	0.9999
Wachtel	0.9999	0.9999	1.0

DISCUSSION

The TBSAs of growing ~four-month-old male and female Hanford miniature swine were calculated using body weight at 0, 8, 12, and 18 weeks according to the methods of Spector (1956). Changes in TBSA were compared to a dermal application area (DAA), which were 5 cm X 5 cm (25 cm^2) at the beginning of study. Changes in DAA, TBSA, and body mass are relevant to correlating dose applied and delivered (systemic exposure) in transdermal drug uptake studies. If the DAA fails to grow proportionally to the body surface area or to body mass, then the dose delivered on a kg or surface area basis may vary across time. Methods for calculating the body surface area of domestic swine include: Meeh (1879), Voit (1901), Rubner (1902), Hogan and Skouby (1923), Brody (1928), Deighton (1932), Kleiber (1947), Spector (1956), and Wachtel (1972). The Spector method is highly respected, hence why it was selected and applied to miniature swine. Wachtel et al. (1972) have suggested that the formula for TBSA derived for domestic swine are not applicable to miniature swine. The Wachtel equation SA (M^2) = 0.121 W(.575) was suggested to be a more accurate, quick assessment of the TBSA of miniature swine. The Wachtel method was compared, as was Brody's equation (SA cm^2 = 970 W.633), to the Spector Method results.

Using the Spector formula for TBSA, the mean DAA/TBSA ratio for Weeks 0 and 8 (N = 32) for a control application area was 0.46% ± 0.04 and 0.51% ± 0.06 (Mean ± SD), respectively. After Week 8, subsequent periodic measurements of the ratio (application area to total body surface area) in 20 kg to 35 kg miniature swine remained steady or essentially unchanged, suggesting proportional changes in growth of both DAA and TBSA (**Table 1**).

The Brodie (**Table 2**) and Wachtel (**Table 3**) methods resulted in slightly different absolute TBSA and ratio values. But, in the end, all three methods were moving proportionally (**Table 4** and **Figures 1 and 2**) over the four measurement time periods. The three alternate methods for TBSA calculation were comparable (<10% difference) with a high correlation coefficient >0.99 (**Table 5**).

CONCLUSIONS

Comparable TBSA and DAA/TBSA ratio data suggested the Spector method was a valid choice. The correlation of the three TBSA calculation methods was >0.99. No actual TBSA reference value or whole body skin area measurements were available to ascertain the accuracy and precision of the formulas.

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