A Retrospective Analysis of Housing Condition-Related Effects in Two-Year Rat Carcinogenicity Studies

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ABSTRACT

Rodent toxicology studies have historically been performed in wire-bottom cages. However, both the National Research Council (NRC) and Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) International, recommend housing rats in solid-bottom cages with bedding to prevent development of foot lesions and/or stress responses. A retrospective analysis of Sprague-Dawley rat carcinogenicity study data (n=25 control groups) compared the effects of individual vs. pair housing and wire-bottom vs. solid-bottom housing on body weight, food consumption, survival, incidence of foot lesions and tumor rate. Survival was analyzed by the Cox proportional hazards model; body weight was analyzed by the Gompertz non-linear mixed model; food consumption was analyzed by the mixed effects ANOVA Model; and the prevalence of foot lesions and tumor rate were analyzed by the Chi-squared test.

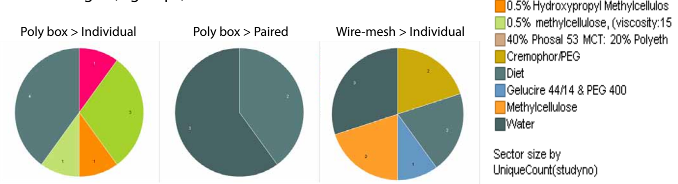
Average body weights were higher for rats housed individually in solid-bottom cages vs. than those housed individually in wire-bottom cages. These body weight differences became significant around Week 25 in males and Week 45 in females, and continued until the end of study (Week 104). At Week 104, the average difference in males was about 50 g or 5% and in females was about 55 g or 11%. The differences in average body weight did not correspond with a significant difference in average food consumption or in survival rates. Compared to the rats housed in wire-bottom cages, rats in solid-bottom cages have lower foot lesion incidence in males but higher incidence in females. The latter result suggests >10% increases of body weight contributes to the formation of foot lesions in females. The total tumor rates for rats housed individually in solid-bottom cages are higher than rats housed individually in wire-bottom cages. This result corresponds with the significant increase in average body weight.

For pair-housed rats in solid-bottom cages compared to individually-housed rats in solid-bottom cages, average food consumption was lower (~7% in males; ~8% in females). The difference in average food consumption corresponded with a lower average body weight (Week 104, ~3% in males, ~9% in females), significantly lower incidence of foot lesions (Week 104, ~12% in males, ~19% in females), a slight improvement in survival (Week 104, ~15% in females only), and significantly lower decreased overall tumor rate (~7% in females only).

In conclusion, this analysis demonstrated that housing conditions (individual vs. paired and wire-bottom vs. solid-bottom) do affect body weight, food consumption, survival, foot lesion and tumor rates in two-year Sprague-Dawley rat carcinogenicity studies. The results provided a contrasting backdrop to a widely accepted dogma that solid-bottom housing is generally better than wire-bottom housing without consideration of individual vs. pair housing. Additionally, regarding solid-bottom housing, there are clear advantages to pair-housing compared to individual housing which may lead to lower food consumption and average body weight, decreased incidence of foot lesions, decreased total tumors rates in females, and increased survival in females.

MATERIALS & METHODS

- Study Details:
  - — Studies initiated 2006 to 2011
  - — 25 control groups, 60 to 80 animals/sex/group
- Housing:
  - — Individually housed in wire-bottom cages (10 groups)
  - — Individually housed in solid-bottom polycarbonate cages (10 groups)
  - — Paired housed in solid-bottom polycarbonate cages (5 groups)
- Feed: Lab Diet 5002, PMI Nutrition International, supplied ad libitum
- Routes of administration: Oral gavage or dietary

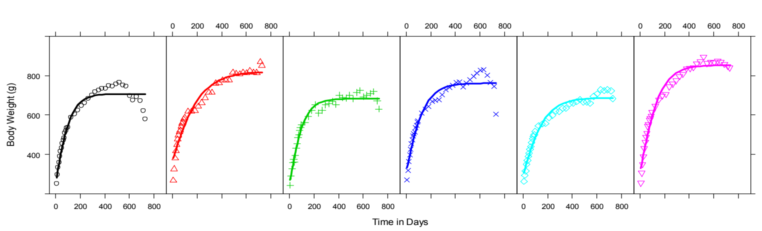


Survival data were analyzed by the Cox proportional hazards model with housing/cage condition treated as a three-level fixed factor.
- The PHREG procedure in SAS 9.2 software was used to fit the Cox model.
- The predicted survival probability was generated for each housing/cage condition.

Food Consumption (Define as the average food consumption across all collection time points for each animal), were analyzed by the Mixed Effects ANOVA Model with housing condition as three-level fixed factor and study(group) as a random factor.
- The MIXED procedure in SAS 9.2 software was used to fit the Mixed Effects ANOVA Model.

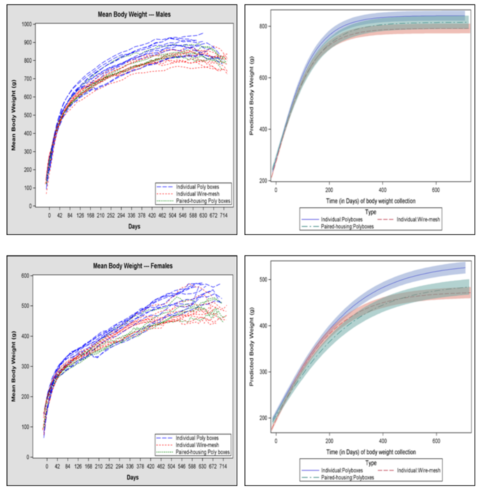
The incidence of foot lesions and tumor rate were analyzed by the Chi-squared test.

Longitudinal body weight were used in the body weight curve fitting.
A three parameter Gompertz non-linear mixed model was used to fit the growth curves for all animals.
- The study(group) was treated as random to account for inter-study variation due to initial body weight difference.
- The NLMIXED procedure in SAS 9.2 software was used to fit the Gompertz model.
- The predicted body weight growth curve was generated for each housing/cage condition.
- Examples of curve fitting with the Gompertz non-linear mixed model are shown below:



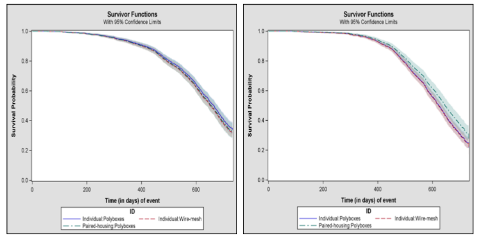
RESULTS

Figure 1. Male and Female Body Weight Summary Curve (Left) and Gompertz Non-Linear Model Output (Right)



- For male animals, at around week 25, the mean body weights began to differ between the solid-bottom and the wire-bottom groups. On average, the body weight of rats in the individual solid-bottom group was about 50 g higher than the average weight of wire-bottom group, and the differences appear to be constant from week 35 to week 104 (~5%). The mean body weight of the pair-housed solid-bottom group was between the other two groups.
- For female animals, at around week 45, the mean body weights began to differ between the solid-bottom groups and the wire-bottom group. On average, the body weight of rats in the individual solid-bottom group was higher than the average weight of wire-bottom group. At the end of the study in Week 104, the differences are about 55 g (~11%). The mean body weight of the pair-housed solid-bottom group was similar to that of the wire-bottom group. The differences in body weight continued to increase after Week 45.

Figure 2. Survival Summary in Prediction Mode for Male and Female Rats

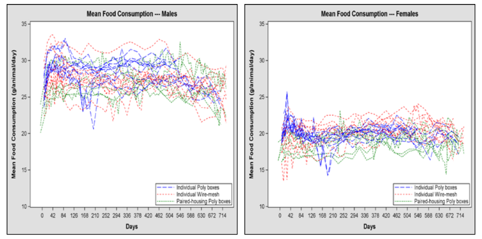


Comparisons	P Value
Individual: Solid vs. Individual: Wire	0.3199
Individual: Solid vs. Paired: Solid	0.5707
Paired: Solid vs. Individual: Wire	0.7921

Comparisons	P Value
Individual: Solid vs. Individual: Wire	0.8586
Individual: Solid vs. Paired: Solid	0.0401
Paired: Solid vs. Individual: Wire	0.0237

- Rats pair-housed in solid-bottom cages had the highest survival rate among all three groups in females.

Figure 3. Summary of Mean Food Consumption



Least Squares Means	
Individual: Solid	28.65
Individual: Wire	27.74
Paired: Solid	26.62

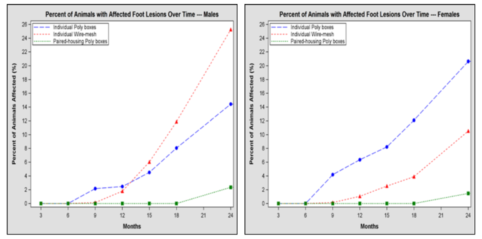
Least Squares Means	
Individual: Solid	20.07
Individual: Wire	20.10
Paired: Solid	18.49

Comparisons	P Value
Individual: Solid vs. Individual: Wire	0.2419
Individual: Solid vs. Paired: Solid	0.0391
Paired: Solid vs. Individual: Wire	0.2383

Comparisons	P Value
Individual: Solid vs. Individual: Wire	0.9491
Individual: Solid vs. Paired: Solid	0.0066
Paired: Solid vs. Individual: Wire	0.0058

- Pair-housed animals consumed the least amount (~8%) of food in all three housing conditions in females; and consumed less food (~7%) compared to the individually housed solid-bottom groups

Figure 4. Summary of Foot Lesion Incidence



- Pair-housed animals had the lowest incidence in foot lesions among all three groups
- Individually housed males in wired cages had the highest rate of foot lesions. This is consistent with the expectation
- Individually housed females in solid bottom cages had the highest rate of foot lesions, suggested >10% increase of body weight contributes to the formation of foot lesions.

Table. Summary of General Tumor Incidence

Tumor Rates in Males(%)	
Individual: Solid	81.6
Individual: Wire	76.9
Paired: Solid	77.9

Tumor Rate in Females (%)	
Individual: Solid	97.2
Individual: Wire	92.8
Paired: Solid	90.0

Comparisons	P Value
Individual: Solid vs. Individual: Wire	0.0472
Individual: Solid vs. Paired: Solid	0.1759
Paired: Solid vs. Individual: Wire	0.7143

Comparisons	P Value
Individual: Solid vs. Individual: Wire	0.0004
Individual: Solid vs. Paired: Solid	<0.0001
Paired: Solid vs. Individual: Wire	0.1349

- Heavier rat correlated with higher prevalence of tumor in both males and females.

RESULTS CONTINUED

Table. Summary of Specific Tumor Incidence

Organ (Male)	Tumor Name	Individual Wire (N=545)		Individual Solid (N=645)		Paired Solid (N=340)	
		#Animal	Rate (%)	#Animal	Rate (%)	#Animal	Rate (%)
Adrenal Glands	Adenoma, Cortical	8	1.47	7	1.09	6	1.76
Brain	Astrocytoma	14	2.57	7	1.09	5	1.47
Parathyroid Glands	Adenoma	12	2.2	11	1.71	4	1.18
Skin	Keratoacanthoma	10	1.83	12	1.86	7	2.06
Thyroid Gland	Adenoma, Follicular Cell	8	1.47	12	1.86	9	2.65
Liver	Adenoma, Hepatocellular	9	1.65	17	2.64	5	1.47
Pancreas	Carcinoma, Islet Cell	12	2.2	8	1.24	13	3.82
Adrenal Gland	Pheochromocytoma	9	1.65	55	8.53	8	2.35
Pancreas	Adenoma, Islet Cell	62	11.38	65	10.08	29	8.53
Adrenal Glands	Pheochromocytoma	74	13.58	41	6.36	54	15.88
Thyroid Gland	Adenoma, C-cell	60	11.01	83	12.87	39	11.47
Pituitary Gland	Adenoma, Pars Distalis	321	58.9	397	61.55	207	60.88

Organ (Female)	Tumor Name	Individual Wire (N=545)		Individual Solid (N=645)		Paired Solid (N=340)	
		#Animal	Rate (%)	#Animal	Rate (%)	#Animal	Rate (%)
Adrenal Glands	Adenoma, Cortical	7	1.28	8	1.24	6	1.76
Uterus with Cervix	Granular Cell Tumor	9	1.65	7	1.09	11	3.24
Vagina	Granular Cell Tumor	8	1.47	13	2.02	12	3.53
Uterus with Cervix	Polyp, Stromal	10	1.83	17	2.64	11	3.24
Pancreas	Adenoma, Islet Cell	15	2.75	16	2.48	10	2.94
Adrenal Gland	Pheochromocytoma	16	2.94	15	2.33	11	3.24
Mammary Gland	Adenoma	24	4.4	13	2.02	11	3.24
Pituitary Gland	Carcinoma, Pars Distalis	17	3.12	23	3.57	16	4.71
Thyroid Gland	Adenoma, C-cell	44	8.07	63	9.77	43	12.65
Mammary Gland	Adenocarcinoma	130	23.85	180	27.91	110	32.35
Mammary Gland	Fibroadenoma	183	33.58	264	40.93	143	42.06
Pituitary Gland	Adenoma, Pars Distalis	423	77.61	515	79.84	245	72.06

- Tumor incidence of pituitary glands in female animals share the same pattern as general tumor incidence. However, no significant difference per Peto test.

CONCLUSIONS

- Housing conditions (individual vs. paired and wire-bottom vs. solid-bottom) impact body weight, food consumption, survival, foot lesions and tumor rates in two-year Sprague-Dawley rat carcinogenicity studies.
- For rats housed individually: Solid-bottom cage has no benefit to SD rats for the endpoints evaluated.
  - — Gained more body weight
  - — Higher tumor incidence
  - — No improvement in survival or incidence of foot lesions (worse in females)
- For rats housed in solid-bottom cages: Pair-housed rats performed better for the endpoints evaluated
  - — Consumed less food and gained less body weight
  - — Lower incidence of foot lesions
  - — Decreased total tumor rates in females
  - — Slightly increased survival (females only).

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DISCLOSURES

AbbVie Inc. sponsored the study. AbbVie contributed to the study design, analysis, data collection, interpretation of data, and writing, reviewing, and approving of the publication. Dong Zhao, Donna Davila, and Ronnie Yeager are employees of AbbVie. Lei Shu was an employee of AbbVie, Inc when the poster was created and approved; She is now an employee of Astellas Pharma US, Inc. MPI Research was contracted by AbbVie to conduct the nonclinical studies which generated part of the data in this publication, and was also involved in the discussion, reviewing, and approving of the publication. Chris Papagiannis, Duane Poage, Janice Hiner, and Daniel Patrick are employees of MPI Research; they have not received any personal compensation from AbbVie.