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July 2, 2015

Dear Dr. Thompson,

Attached is a report entitled "Pathology Peer Slide Review of Non-neoplastic Finding in the Duodenum of Mice and Rats Treated with SDD in ACC-TS and NTP studies." This report involves reviews of histologically-prepared duodenal specimens from studies in which F344/N rats and 86C3F1 mice were treated with varying doses of sodium dichromate dihydrate (SDD). We reviewed selected slides from two sets of studies, one conducted by The American Chemistry Council (ACC) and ToxStrategies at Southern Research Institute, and one conducted by the National Toxicology Program. The attached report represents our consensus opinions.

Sincerely,

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Pathology Peer Slide Review of Non-neoplastic Findings in the Duodenum of Mice and Rats Treated with SDD in ACC-TS and NTP Studies

INTRODUCTION

In 2008, the National Toxicology Program released a technical report that described the conduct and results of 2-year studies in which sodium dichromate dihydrate (SDD, hexavalant chromium, CrVI) were administered by drinking water to F344/N rats and B6C3F1 mice (NTP, 2008). A summarized version of those results was also published in the peer-reviewed literature (Stout et al., 2009). Among treatment-related histopathologic results reported for male and female mice were increased incidences of benign and malignant neoplasms of the duodenum, plus non-neoplastic findings in the duodenum that included diffuse epithelial hyperplasia and histiocytic cellular infiltration. Focal epithelial hyperplasia was also observed in the duodenum of mice, and although the incidence of this finding was not statistically significant, and there was no dose-dependent relationship, it was considered to be a pre-neoplastic precursor lesion. Unlike mice, rats exposed to SDD in the NTP studies did not develop duodenal neoplasia, nor was focal epithelial hyperplasia reported, and the sole treatment-related non-neoplastic histopathologic finding in the duodenum was the presence of histiocytic cellular infiltrates. Also referenced in this report were the results of a 3--month exposure study (NTP, 2007), in which duodenal findings consisted of increased incidences of epithelial hyperplasia in mice, and histiocytic cellular infiltration in both rats and mice. There were qualitative differences between the histopathologic results of these NTP trials and those of 90-day rat and mouse studies conducted subsequently by The American Chemistry Council (ACC) and ToxStrategies at Southern Research Institute (SRI, 2011a and SRI, 2011b) regarding the diagnosis of duodenal findings. Such differences occurred despite the fact that the same rodent strains and comparable SDD dose concentrations were used in both the NTP and ACC-TS studies. Treatment-related histopathologic findings in the ACC-TS 90-day mouse study (60, 170, and 520 mg/L SDD dose groups) included crypt epithelial hyperplasia, histiocytic cellular infiltration of the villous lamina propria, cytoplasmic vacuolization of the villous epithelium, multinucleated syncytia of the villous lamina propria, villous atrophy, and apoptosis. The ACC-TS experiments also featured an additional sacrifice at Day 8, at which time point treatment-related findings included cytoplasmic vacuolization of the villous epithelium in the 170 and 520 mg/L SDD dose groups, and crypt epithelia hyperplasia and villous atrophy of mice in the 520 mg/L SDD dose group.

The primary purpose of this task was to investigate the reason(s) why the histopathologic results of the subchronic (90 day) NTP and ACC-TS studies differed. Possible causes include: 1) inter-study variation in animal responses; 2) subtle differences in experimental design and/or conduct; and 3) differences in pathologist interpretation of morphologic observations. Resolution of differences between these two sets of studies may provide information pertinent to the carcinogenic mode of action of CrVI.

METHODS

Selected NTP and ACC-TS slides of duodenum were evaluated jointly by two independently contracted ACVP board-certified veterinary pathologists, Drs. John M. Cullen (North Carolina State University) and Jerrold M. Ward (Global VetPathology). The specific goals of this review were to determine: 1) the degree to which histopathologic duodenal findings in the NTP and ACC-TS studies were qualitatively different or similar; 2) the degree to which findings in mice

were qualitatively different or similar to those observed in rats; 3) whether the various types of findings are likely representative of a single pathologic process versus independent responses.

Materials examined include selected slides of duodenum from: 1) ACC-TS 90-day (subchronic) and 8-day studies of SDD in female mice and rats; 2) NTP 90-day and 2-year (chronic) studies of SDD in male and female mice and rats. Slides for examination were selected impartially and consecutively. Exceptions included slides in which there was substantial autolytic change in the sections, or missing slides, in which cases the next consecutive slides were selected. A single section of duodenum was available and examined per animal. A summary of the different studies and numbers of slides reviewed for each is presented in Table 1.

Table 1. Overview of Pathologist Slide Review of Duodenal Sections from NTP and ACC-TS Studies

Study Site	Species	Sacrifice(s)	Sex	Dose Groups Evaluated (mg/L)	Total Number of Animals Examined
NTP	Mouse	90 days	M	0, 1000	20
		90 days	F	0, 1000	20
		2 year	M	0, 14.3, 28.6, 85.7, 257.4	51
		2 year	F	0, 14.3, 57.3, 172, 516	50
	Rat	90 days	M	0, 500, 1000	30
		90 days	F	0, 1000	20
		2 year	M	0, 57.3, 172, 516	43
		2 year	F	0, 172, 516	41
ACC-TS	Mouse	90 days	F	0, 60, 170, 520	40
	Rat	8 days	F	170, 520	11
		90 days	F	0, 170, 520	30
				Grand Total	356

The two pathologists initially performed a brief qualitative evaluation of slides from the ACC-TS and NTP studies to establish the most appropriate diagnostic criteria and terminology to be used for the review. The final lexicon was derived from the experience of the pathologists, current convention, and consultation of the scientific literature.

The pathologists subsequently reviewed the selected slides via brightfield microscopy without knowledge of the prior individual animal diagnoses. Findings were scored for severity according to the following system: Grade 0 = not remarkable; Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate. Severe changes were not observed. Findings were tabulated on Excel spreadsheets.

RESULTS

Following the initial slide review, it was determined that potential treatment-related non-neoplastic findings in the duodenum of mice and rats were limited to the following five diagnoses: 1) Villus, histiocytic cellular infiltrates; 2) Villus, atrophy/blunting; 3) Villus, enterocyte vacuolation; 4) Villus, single cell necrosis; 5) Crypt, epithelial hyperplasia. With rare exceptions, such findings were not present in control animals, and the prevalence and/or severity of these findings often increased in a dose-responsive manner in SDD-treated animals.

Histopathologic findings are illustrated in photomicrographic Figures 1-8 (all figures are H&E). Histiocytic infiltration occurred primarily in the lamina propria of the villus tips, and was characterized by small nodular aggregations of histiocytic macrophages with abundant, faintly granular, eosinophilic cytoplasm. Some macrophages formed multinucleated syncytia. Villi were considered atrophied/blunted when they appeared shortened and /or thickened relative to those of control animals. Enterocyte vacuolation appeared as single or multiple, sharply defined, clear or slightly flocculent spaces within the apical cytoplasm of terminal villus enterocytes. Single cell necrosis appeared either as cells with condensed cytoplasm and smudged nuclei with a peripheral halo (apoptotic appearance), or more often as cells with karyorrhectic nuclei, that were located either within the villus epithelium or lamina propria. It could not always be readily determined whether such cells were originally enterocytes or inflammatory cells. Crypt epithelial cell hyperplasia was characterized by elongated crypts that were lined by increased numbers of crowded tall enterocytes with hyperchromatic basophilic cytoplasm and nuclear chromatin clumping. In more extensively affected cases (mice especially), the enterocytes of villi additionally displayed increased cell height and tinctorial changes compared to those of controls.

ACC-TS Female Mice versus ACC-TS Female Rats at 90 Days (Tables 2 and 3)

Similar types of findings were present in both ACC-TS mice and rats at 90 days, except that enterocyte vacuolation was not evident in rats. Overall, the prevalence and severity of duodenal findings were greater in mice than in rats.

ACC-TS Female Mice versus NTP Female and Male Mice at 90 Days (Tables 2, 5 and 6)

The prevalence and severity of findings in ACC-TS female mice were generally greater than those of NTP female mice, despite the fact that NTP females received almost double the dose of SDD (ACC-TS = 520 mg/L, NTP = 1000 mg/L). Enterocyte vacuolation was not present in NTP female mice. However, the prevalence and severity of all five findings were comparable between NTP male mice and ACC-TS female mice at the respective highest doses.

ACC-TS Female Rats versus NTP Female and Male Rats at 90 Days (Tables 3, 7, and 8)

The prevalence and severity of histiocytic cellular infiltrates and crypt epithelial hyperplasia were comparable between ACC-TS female rats and NTP female and male rats at their respective highest doses (ACC-TS = 520 mg/L, NTP = 1000 mg/L). However, atrophy/blunting and single cell necrosis were not observed in NTP female rats, despite a high and modest prevalence of those findings, respectively, in ACC-TS female rats. Enterocyte vacuolation was not observed in female or male rats from either test site.

NTP Female Mice versus NTP Male Mice versus NTP Female Rats versus NTP Male Rats at 90 Days (Tables 5, 6, 7, and 8)

At the highest SDD dose of 1000 mg/L, the prevalence and severity of findings were generally greater in mice than in rats, and were generally greater in males than in females. Thus male mice exhibited the greatest prevalence and severity of findings, whereas female rats exhibited the lowest. For example, while modest to high prevalences of atrophy/blunting, enterocyte vacuolation, and single cell necrosis were present in NTP male mice, those findings were completely absent in NTP female rats. However, histiocytic cellular infiltrates and crypt epithelial hyperplasia were always observed to some degree in both species and sexes of NTP rodents that were treated with 1000 mg/L at 90 days.

ACC-TS Female Rats at 8 Days versus ACC-TS Female Rats at 90 Days (Tables 3 and 4)

At identical doses of 520 mg/L, the prevalence and severity of atrophy/blunting and crypt epithelial hyperplasia, and the prevalence of enterocyte vacuolation (which were low), were greater in ACC-TS female rats at 8 days than at 90 days.

On the other hand, histiocytic cellular infiltrates, which were observed in 9/10 female rats at 90 days, were not present to any degree in female rats sacrificed at 8 days. The prevalence of single cell necrosis was low at both time points and only slightly greater in ACC-TS 90 day females.

NTP Female and Male Mice at 2 Years versus 90 Days (Tables 5, 6, 9, and 10)

For male mice of the highest respective dose groups (90 day = 1000 mg/L, 2 year = 257.4 mg/L), the prevalence of findings was generally comparable or slightly greater in animals sacrificed at 90 days, but the severity of findings tended to be greater at 2 years. For female mice of the highest respective dose groups (90 day = 1000 mg/L, 2 year = 516 mg/L), both the prevalence and severity of findings were generally greater at 2 years. For example, while the prevalence of enterocyte vacuolation and single cell necrosis were at or near zero in 90-day females of the highest dose group, each of those findings were present in 7 of 10 high-dose female mice at 2 years.

NTP Female and Male Rats at 2 Years versus 90 Days (Tables 7, 8, 11, and 12)

For male rats of the highest respective dose groups (90 day = 1000 mg/L, 2 year = 516 mg/L), the prevalence and severity of findings were generally comparable at 90 days versus 2 years with one notable exception: atrophy/blunting was not observed in 90 day male rats but minimal to mild atrophy/blunting was present in 7 of 11 males at 2 years. For female rats of the highest respective dose groups (90 day = 1000 mg/L, 2 year = 516 mg/L), the prevalence and severity of findings was also comparable at 90 days versus 2 years. However, although atrophy/blunting and enterocyte vacuolation were not evident at 90 days, both of these findings were present (albeit low prevalence and severity) in high-dose females at 2 years.

DISCUSSION

Overall, the non-neoplastic effects of SDD administration on the duodenum were qualitatively similar among both mice and rats in both the ACC-TS and NTP studies, and these included primarily four findings that involved villi (histiocytic cellular infiltrates, atrophy/blunting, enterocyte vacuolation, and single cell necrosis) plus one finding that involved crypts (epithelial hyperplasia). However, the degree (prevalence and severity) to which these findings were evident varied according to the study (i.e., NTP vs. ACC-TS), species and sex of the animals, and the duration of exposure prior to sacrifice. In isolation, the SDD concentration appeared to be less of a factor when comparisons were made using the highest dose groups; for example, the prevalence and severity of findings in ACC-TS female mice were greater than those of NTP female mice at 90 days, despite the fact that NTP females received almost double the concentration of SDD[1]. In general, the prevalence and severity of findings tended to be greater in mice versus rats, males versus females, ACC-TS animals versus NTP animals, and at 2 years versus 90 days. It should be noted that each of the five major findings was identified to some degree in both rats and mice from at least one sacrifice time point and test site, although findings of enterocyte vacuolation, single cell necrosis, and to a lesser degree, atrophy/blunting, were frequently less prevalent or inapparent in rats versus mice.

When comparing the ACC-TS and NTP 90-day studies, it became apparent that there were actual inter-site differences in the degree to which the non-neoplastic duodenal effects occurred in rats and mice. One possible reason for this is that the sources of the rat strain (F344 from Taconic or Charles River) and mouse hybrid line (Taconic or Charles River) differed between the NTP and ACC-TS studies. Thus strain variation may have contributed to some degree to qualitative

[1] Water/test article consumption differed between the studies and thus the mg/kg doses were generally higher in the ACC-TS studies.

or quantitative differences between the studies. However, there were also qualitative interpretive differences between the results of the current review and those of the original ACC-TS and NTP reports. For example, the original ACC-TS report contained diagnoses of "multinucleated syncytia of the villous lamina propria", but that particular finding could not be differentiated from histiocytic cellular infiltrates during this review. Diagnostic differences between the current review and the reported results of the NTP 90 day study (NTP, 2007) were more obvious. For example, the original NTP report contained findings of only histiocytic cellular infiltration and epithelial hyperplasia for male and female mice, and only histiocytic cellular infiltration for male and female rats. In the text of that report it was noted that "epithelial cells lining the tips of the villi of many of the exposed mice were swollen and had vacuolated cytoplasm", but that observation was not assigned a diagnosis (e.g., enterocyte vacuolation). In contrast, the current review of NTP male mice at 90 days identified minimal to moderate atrophy/blunting of villi in 10 of 10 mice minimal to mild enterocyte vacuolation in 9 of 10 mice, and minimal single cell necrosis of villi in 4 of 10 mice. The current review also identified minimal to mild crypt epithelial hyperplasia in 7 of 10 NTP male rats at 90 days, and minimal crypt epithelial hyperplasia in 3 of 10 NTP female rats.

Referring to the non-neoplastic findings in the duodenum of mice, the NTP 90-day study report (NTP, 2007) stated that "Collectively, these duodenal lesions suggest regenerative hyperplasia secondary to previous epithelial cell damage or degeneration." An almost identical statement was reiterated in the 2-year NTP study report (NTP, 2008). The notion that crypt epithelial hyperplasia occurred as a sequelae to villus epithelial cell damage is supported by several lines of evidence that were apparent from the current review: 1) findings suggestive of villus epithelial injury such as atrophy/blunting, enterocyte vacuolation, and single cell necrosis; 2) the complete absence of histopathologic findings indicative of crypt epithelial cell damage; and 3) the likelihood that mucosal changes would have been far more profound had the regenerative crypt compartment been affected in lieu of, or in addition to, the villus compartment (e.g., as in canine parvoviral enteritis). Although it is theoretically possible that crypt epithelial hyperplasia might have resulted from direct chemically-induced mitogenic stimulation, this is to some extent belied by the frequently observed finding of atrophied and blunted villi. Duodenal villous atrophy is thought to occur as a consequence of either damage to enterocytes or defective epithelial regeneration (Shalimar et al., 2013), the latter of which did not appear to occur in the ACC-TS or NTP studies based on consistent diagnoses of crypt epithelial hyperplasia. Instances in which villous atrophy/blunting were not observed in these studies (e.g. NTP rats at 90 days), could be attributed to the homeostatic ability of regenerative crypt epithelial hyperplasia to fully compensate for villus epithelial cell loss that was comparatively less severe. Similarly, the fact that findings of enterocyte vacuolation and single cell necrosis of the villus tips were often present at low prevalence (or were non-existent) in these studies does not mean that such events did not contribute to villous epithelial damage. Lower degrees of enterocyte damage at the villus tips may not always be morphologically evident because of the transient nature of these cells as they perpetually exfoliate into the intestinal lumen. For example, a single mouse villus is reported to exfoliate approximately 1,400 enterocytes into the intestinal lumen per 24 hour period (Potten and Loeffler, 1990). However, in contrast to the minor degrees of enterocyte vacuolation and single cell necrosis that were present in at least some SDD-treated animals, it is important to note that such findings were not observed to any degree in any of the 105 control animals examined during this review.

Histiocytic cellular infiltration of the distal villous lamina propria was observed consistently in the overwhelming majority of high dose mice and rats examined during this review, with the notable exception of ACC-TS rats sacrificed at 8 days. The precise reason for the presence of these cells is undetermined, and authors of the original ACC-TS and NTP studies did not propose any mechanistic theories (SRI, 2011a; SRI, 2011b; NTP, 2007; NTP, 2008). In the NTP studies, morphologically similar histiocytic cellular infiltrates were additionally observed in the liver, and mesenteric and/or pancreatic lymph nodes (NTP, 2007; NTP, 2008). While it is possible that such infiltrates were recruited to the villus tips

in order to scavenge the residual cellular components of damaged enterocytes and other cell populations (e.g., resident leukocytes), there is insufficient evidence available to support this hypothesis.

SUMMARY AND CONCLUSIONS

Findings in mice and rats of the ACC-TS and NTP 90-day studies were qualitatively similar, although the degree to which animals were affected varied according to the study (i.e., NTP vs. ACC-TS), species and sex of the animals, and the duration of exposure prior to sacrifice. The primary potential treatment-related findings included histiocytic cellular infiltrates within the villous lamina propria, atrophy/blunting of villi, enterocyte vacuolation of villi, single cell necrosis of villi, and crypt epithelial hyperplasia. In general, the prevalence and severity of findings tended to be greater in mice versus rats, males versus females, and in ACC-TS animals versus NTP animals. However, based on the qualitative similarity of lesions in mice and rats in the examined studies, it appears likely that at least several of these findings (atrophy/blunting, enterocyte vacuolation, single cell necrosis, and crypt epithelial hyperplasia) were pathogenically inter-related; i.e., they portray a process in which chemically-induced damage to villus enterocytes resulted in regenerative crypt epithelial hyperplasia.

There appeared to be two major reasons for the differences in results reported by ACC-TS and NTP for their respective 90-day mouse and rat studies: 1) the prevalence and severity of findings as determined during this review were generally greater in ACC-TS animals versus NTP animals; and 2) there were differences in diagnostic interpretation between the ACC-TS and NTP studies. In particular, the NTP studies did not report diagnoses of atrophy/blunting, enterocyte vacuolation, nor single cell necrosis in mice or rats, nor did they identify any occurrence of crypt epithelial hyperplasia in rats. Because the scope of current review was limited entirely to non-tumor findings in a single target tissue, such interpretive differences could be attributed to the greater level of scrutiny applied to types of morphologic changes that were relatively minor when compared to the identification of neoplastic lesions or findings indicative of systemic toxicity, for example.

Finally, it is reasonable to surmise that the persistent crypt epithelial regenerative response that was induced and maintained by continuous and chronic SDD-induced villus damage may have contributed to the generation of duodenal neoplasms in mice sacrificed at 2 years, and that the lack of similar tumors in rats may have been related to the comparatively less profound duodenal changes observed in that species, in addition to other potential factors (relative inter-species differences in the efficiency of enterocyte DNA repair mechanisms, e.g.).

July 3, 2015

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ADDITIONAL TABLES

Table 2. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of ACC-TS Female Mice at 90 Days									
Study	**Species**	**Sex**	**Sacrifice**	**Site**	**Diagnosis**	**Dose Group (mg/L)**			
						0	**60**	**170**	**520**
ACC-TS	Mouse	F	90 days	Villus	n	10	10	10	10
					Histiocytic Cellular Infiltrates	0	1	10	10
					Grade 1	-	1	2	2
					Grade 2	-	-	8	4
					Grade 3	-	-	-	4
					Atrophy/Blunting	0	4	10	10
					Grade 1	-	4	8	6
					Grade 2	-	-	2	1
					Grade 3	-	-	-	3
					Enterocyte Vacuolation	0	3	8	7
					Grade 1	-	2	6	2
					Grade 2	-	1	2	2
					Grade 3	-	-	-	3
					Single Cell Necrosis	0	0	0	6
					Grade 1	-	-	-	6
					Grade 2	-	-	-	-
					Grade 3	-	-	-	-
				Crypt	n	10	10	10	10
					Epithelial Hyperplasia	0	3	6	7
					Grade 1	-	3	6	2
					Grade 2	-	-	-	3
					Grade 3	-	-	-	2

Table 3. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of ACC-TS Female Rats at 90 Days								
Study	**Species**	**Sex**	**Sacrifice**	**Site**	**Diagnosis**	**Dose Group (mg/L)**		
						0	**170**	**520**
ACC-TS	Rat	F	90 days	Villus	n	10	10	10
					Histiocytic Cellular Infiltrates	0	10	9
					Grade 1	-	9	5
					Grade 2	-	1	4
					Grade 3	-	-	-
					Atrophy/Blunting	0	4	8
					Grade 1	-	4	8
					Grade 2	-	-	-
					Grade 3	-	-	-
					Enterocyte Vacuolation	0	0	0
					Grade 1	-	-	-
					Grade 2	-	-	-
					Grade 3	-	-	-
					Single Cell Necrosis	0	0	3
					Grade 1	-	-	3
					Grade 2	-	-	-
					Grade 3	-	-	-
				Crypt	n	10	10	10
					Epithelial Hyperplasia	0	3	2
					Grade 1	-	3	2
					Grade 2	-	-	-
					Grade 3	-	-	-

Table 4. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of ACC-TS Female Rats at 8 Days							
Study	**Species**	**Sex**	**Sacrifice**	**Site**	**Diagnosis**	**Dose Group (mg/L)**	
						170	520
ACC-TS	Rat	F	8 days	Villus	n	6	5
					Histiocytic Cellular Infiltrates	0	0
					Grade 1	-	-
					Grade 2	-	-
					Grade 3	-	-
					Atrophy/Blunting	6	5
					Grade 1	1	2
					Grade 2	4	3
					Grade 3	1	-
					Enterocyte Vacuolation	1	2
					Grade 1	1	2
					Grade 2	-	-
					Grade 3	-	-
					Single Cell Necrosis	0	1
					Grade 1	-	1
					Grade 2	-	-
					Grade 3	-	-
				Crypt	n	6	5
					Epithelial Hyperplasia	6	5
					Grade 1	1	3
					Grade 2	5	2
					Grade 3	-	-

Table 5. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Male Mice at 90 Days							
						Dose Group (mg/L)	
Study	**Species**	**Sex**	**Sacrifice Day**	**Site**	**Diagnosis**	**0**	**1000**
NTP	Mouse	M	90 days	Villus	n	10	10
					Histiocytic Cellular Infiltrates	0	10
					Grade 1	-	3
					Grade 2	-	7
					Grade 3	-	-
					Atrophy/Blunting	0	10
					Grade 1	-	1
					Grade 2	-	7
					Grade 3	-	2
					Enterocyte Vacuolation	0	9
					Grade 1	-	6
					Grade 2	-	3
					Grade 3	-	-
					Single Cell Necrosis	0	4
					Grade 1	-	4
					Grade 2	-	-
					Grade 3	-	-
				Crypt	n	10	10
					Epithelial Hyperplasia	0	5
					Grade 1	-	4
					Grade 2	-	1
					Grade 3	-	-

Table 6. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Female Mice at 90 Days							
						Dose Group (mg/L)	
Study	**Species**	**Sex**	**Sacrifice Day**	**Site**	**Diagnosis**	**0**	**1000**
NTP	Mouse	F	90 days	Villus	n	10	9
					Histiocytic Cellular Infiltrates	0	9
					Grade 1	-	3
					Grade 2	-	6
					Grade 3	-	-
					Atrophy/Blunting	0	9
					Grade 1	-	5
					Grade 2	-	4
					Grade 3	-	-
					Enterocyte Vacuolation	0	0
					Grade 1	-	-
					Grade 2	-	-
					Grade 3	-	-
					Single Cell Necrosis	0	1
					Grade 1	-	1
					Grade 2	-	-
					Grade 3	-	-
				Crypt	n	10	9
					Epithelial Hyperplasia	0	9
					Grade 1	-	9
					Grade 2	-	-
					Grade 3	-	-

Table 7. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Male Rats at 90 Days								
						Dose Group (mg/L)		
Study	**Species**	**Sex**	**Sacrifice**	**Site**		**0**	**500**	**1000**
NTP	Rat	M	90 days	Villus	n	10	10	10
					Histiocytic Cellular Infiltrates	0	5	10
					Grade 1	-	4	6
					Grade 2	-	1	4
					Grade 3	-	-	-
					Atrophy/Blunting	0	0	0
					Grade 1	-	-	-
					Grade 2	-	-	-
					Grade 3	-	-	-
					Enterocyte Vacuolation	0	0	0
					Grade 1	-	-	-
					Grade 2	-	-	-
					Grade 3	-	-	-
					Single Cell Necrosis	0	ne[a]	1
					Grade 1	-	ne	1
					Grade 2	-	ne	-
					Grade 3	-	ne	-
				Crypt	n	10	10	10
					Epithelial Hyperplasia	0	5	7
					Grade 1	-	5	6
					Grade 2	-	-	1
					Grade 3	-	-	-

[a]ne =not evaluated

Table 8. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Female Rats at 90 Days							
						Dose Group (mg/L)	
Study	**Species**	**Sex**	**Sacrifice**	**Site**		**0**	**1000**
NTP	Rat	F	90 days	Villus	n	10	10
					Histiocytic Cellular Infiltrates	1	9
					Grade 1	1	7
					Grade 2	-	2
					Grade 3	-	-
					Atrophy/Blunting	0	0
					Grade 1	-	-
					Grade 2	-	-
					Grade 3	-	-
					Enterocyte Vacuolation	0	0
					Grade 1	-	-
					Grade 2	-	-
					Grade 3	-	-
					Single Cell Necrosis	0	0
					Grade 1	-	-
					Grade 2	-	-
					Grade 3	-	-
				Crypt	n	10	10
					Epithelial Hyperplasia	0	3
					Grade 1	-	3
					Grade 2	-	-
					Grade 3	-	-

Table 9. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Male Mice at 2 Years										
						Dose Group (mg/L)				
Study	**Species**	**Sex**	**Sacrifice Day**	**Site**	**Diagnosis**	**0**	**14.3**	**28.6**	**85.7**	**257.4**
NTP	Mouse	M	2 year	Villus	n	10	10	11	10	10
					Histiocytic Cellular Infiltrates	0	1	3	9	9
					Grade 1	-	1	3	2	-
					Grade 2	-	-	-	7	8
					Grade 3	-	-	-	-	1
					Atrophy/Blunting	0	0	2	8	8
					Grade 1	-	-	2	6	4
					Grade 2	-	-	-	2	2
					Grade 3	-	-	-	-	2
					Enterocyte Vacuolation	0	0	2	2	6
					Grade 1	-	-	2	1	1
					Grade 2	-	-	-	1	5
					Grade 3	-	-	-	-	-
					Single Cell Necrosis	0	0	3	5	5
					Grade 1	-	-	2	4	2
					Grade 2	-	-	1	1	3
					Grade 3	-	-	-	-	-
				Crypt	n	10	10	11	10	10
					Epithelial Hyperplasia	0	0	3	8	9
					Grade 1	-	-	3	8	1
					Grade 2	-	-	-	-	6
					Grade 3	-	-	-	-	2

Table 10. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Female Mice at 2 Years										
						Dose Group (mg/L)				
Study	**Species**	**Sex**	**Sacrifice Day**	**Site**	**Diagnosis**	**0**	**14.3**	**57.3**	**172**	**516**
NTP	Mouse	F	2 year	Villus	n	9	10	10	10	11
					Histiocytic Cellular Infiltrates	0	0	0	9	10
					Grade 1	-	-	-	8	1
					Grade 2	-	-	-	1	9
					Grade 3	-	-	-	-	-
					Atrophy/Blunting	0	2	1	3	10
					Grade 1	-	2	1	2	7
					Grade 2	-	-	-	1	3
					Grade 3	-	-	-	-	-
					Enterocyte Vacuolation	0	0	1	5	7
					Grade 1	-	-	1	3	4
					Grade 2	-	-	-	2	3
					Grade 3	-	-	-	-	-
					Single Cell Necrosis	0	0	2	3	7
					Grade 1	-	-	2	3	5
					Grade 2	-	-	-	-	2
					Grade 3	-	-	-	-	-
				Crypt	n	9	10	10	10	11
					Epithelial Hyperplasia	0	2	4	8	10
					Grade 1	-	2	4	7	2
					Grade 2	-	-	-	1	7
					Grade 3	-	-	-	-	1

Table 11. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Male Rats at 2 Years									
						Dose Group (mg/L)			
Study	**Species**	**Sex**	**Sacrifice**	**Site**		**0**	**57.3**	**172**	**516**
NTP	Rat	M	2 years	Villus	n	11	11	10	11
					Histiocytic Cellular Infiltrates	0	1	10	11
					Grade 1	-	1	10	3
					Grade 2	-	-	-	7
					Grade 3	-	-	-	1
					Atrophy/Blunting	0	0	2	7
					Grade 1	-	-	2	5
					Grade 2	-	-	-	2
					Grade 3	-	-	-	-
					Enterocyte Vacuolation	0	0	0	1
					Grade 1	-	-	-	1
					Grade 2	-	-	-	-
					Grade 3	-	-	-	-
					Single Cell Necrosis	0	0	0	1
					Grade 1	-	-	-	1
					Grade 2	-	-	-	-
					Grade 3	-	-	-	-
				Crypt	n	11	11	10	11
					Epithelial Hyperplasia	0	0	5	8
					Grade 1	-	-	5	6
					Grade 2	-	-	-	2
					Grade 3	-	-	-	-

Table 12. Prevalence and Severity of Non-neoplastic Findings in the Duodenum of NTP Female Rats at 2 Years								
						Dose Group (mg/L)		
Study	**Species**	**Sex**	**Sacrifice**	**Site**		**0**	**172**	**516**
NTP	Rat	F	2 years	Villus	n	15	10	15
					Histiocytic Cellular Infiltrates	0	6	12
					Grade 1	-	6	8
					Grade 2	-	-	4
					Grade 3	-	-	-
					Atrophy/Blunting	1	0	3
					Grade 1	-	-	2
					Grade 2	-	-	1
					Grade 3	1	-	-
					Enterocyte Vacuolation	0	0	2
					Grade 1	-	-	2
					Grade 2	-	-	-
					Grade 3	-	-	-
					Single Cell Necrosis	0	0	0
					Grade 1	-	-	-
					Grade 2	-	-	-
					Grade 3	-	-	-
				Crypt	n	15	10	15
					Epithelial Hyperplasia	0	0	2
					Grade 1	-	-	2
					Grade 2	-	-	-
					Grade 3	-	-	-

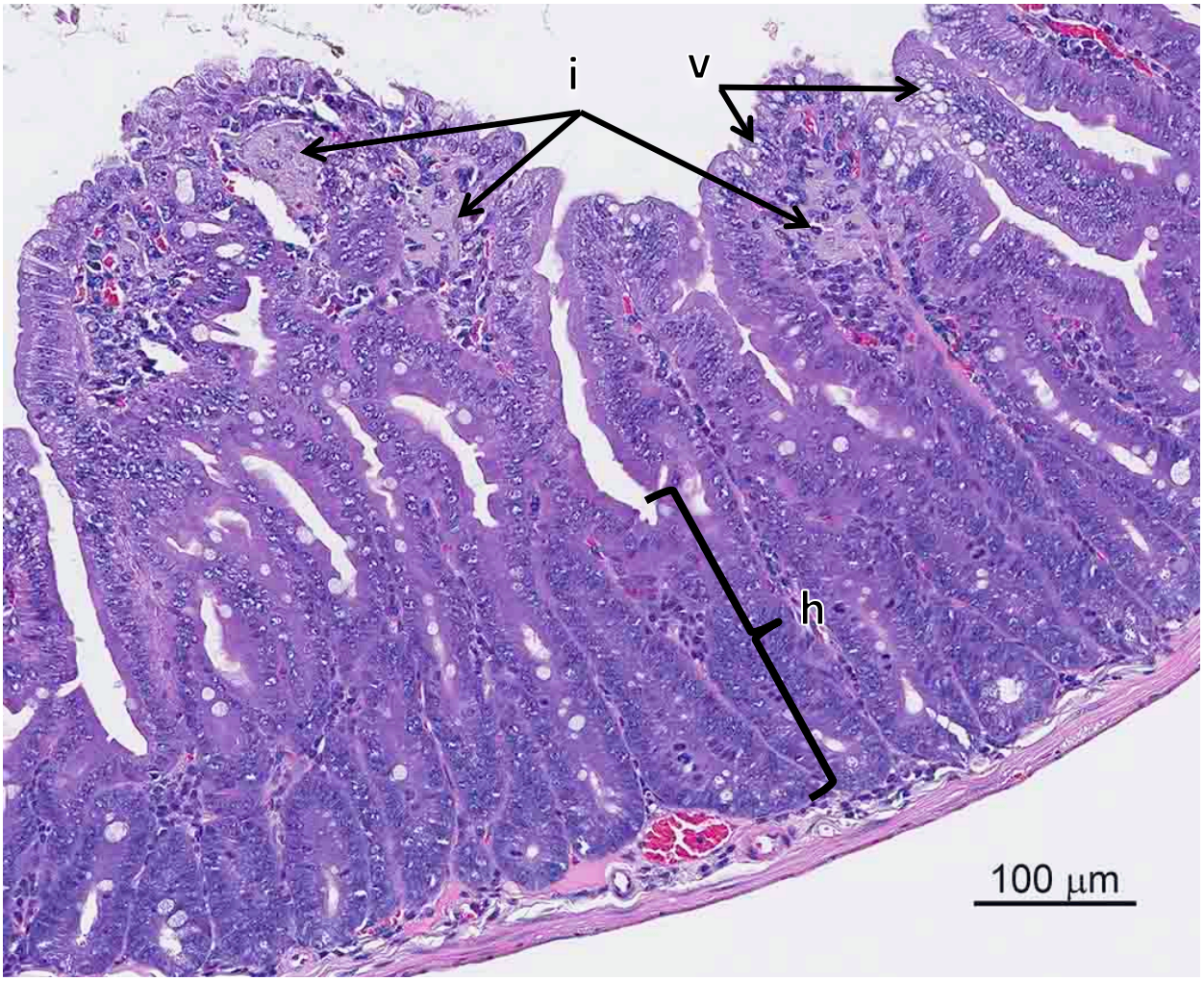

Figure 1. Duodenal findings include moderate histiocytic infiltrates (i), moderate villus atrophy and blunting, moderate villus enterocyte vacuolation (v), minimal single cell necrosis in villi, and moderate crypt epithelial hyperplasia (h). Female mouse exposed to 520 mg/L SDD for 90 days from the ACC-TS study (Animal No. 7F482).

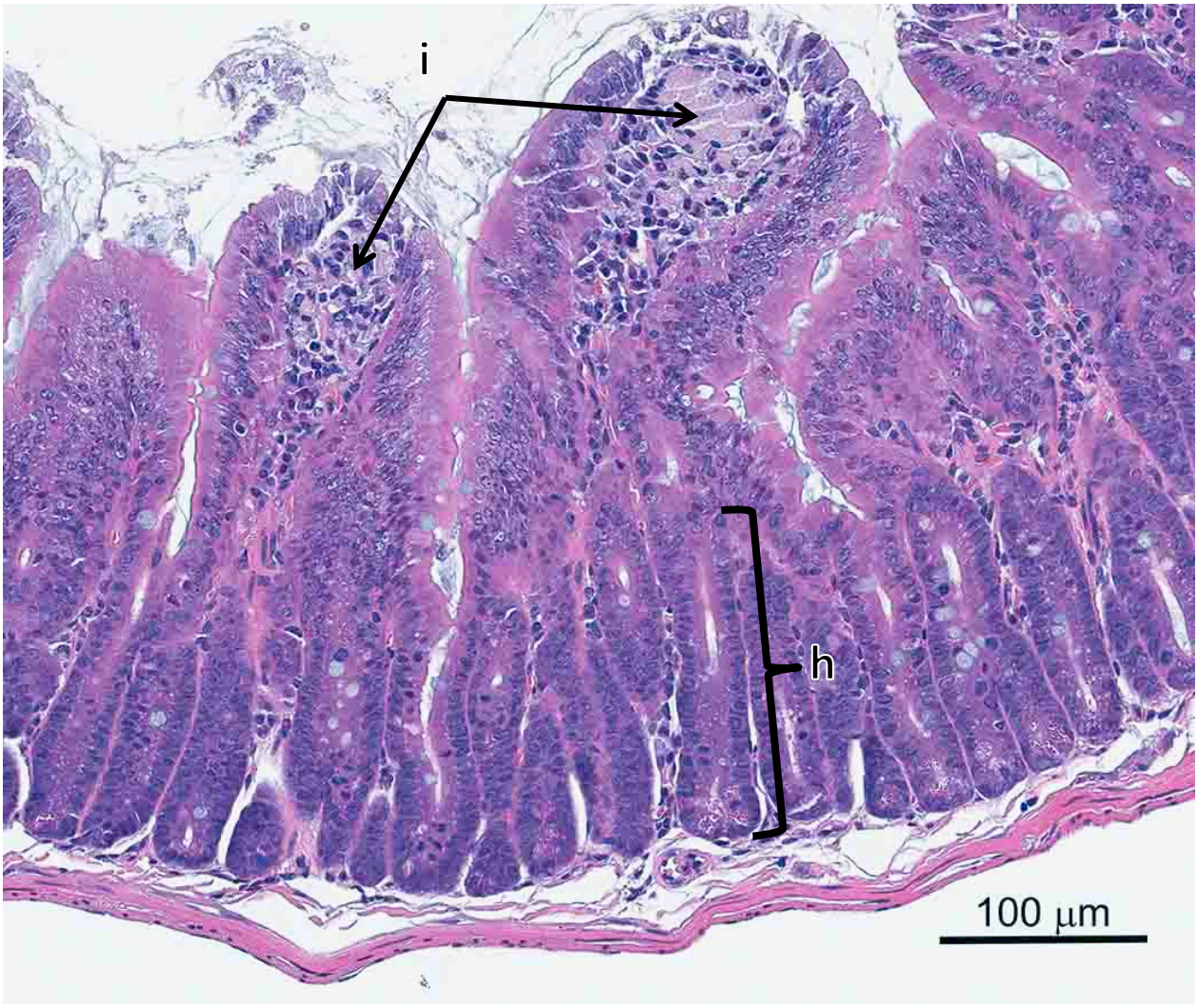

Figure 2. Duodenal villus atrophy and blunting in this NTP mouse are comparable to changes described in the duodenum from the ACC-TS study mouse in the preceding figure, except that crypt epithelial hyperplasia (h) was slightly less florid, and enterocyte vacuolation was not apparent. i = histiocytic infiltrates. Duodenum of a female mouse exposed to 1000 mg/L SDD for 90 days from the NTP study (Animal No. EF112).

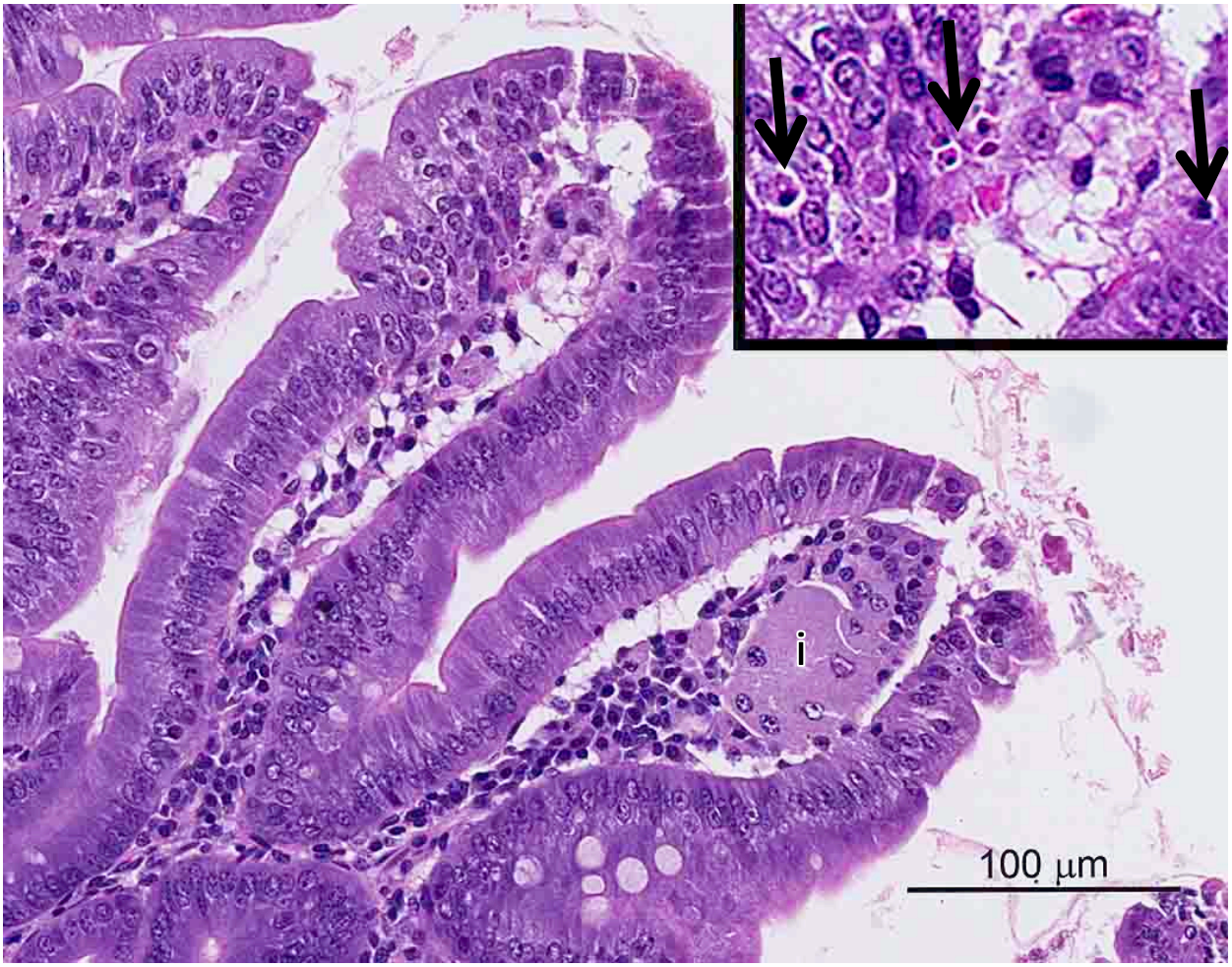

Figure 3. Duodenal single cell necrosis of the villi (arrows) is apparent in this high magnification view and inset. Such necrosis was not observed in the duodenum of control mice. Histiocytic infiltrates (i) occasionally formed syncytia, as in this case. Duodenum of a male mouse exposed to 257.4 mg/L SDD for 2 years from the NTP study (Animal No. OM211).

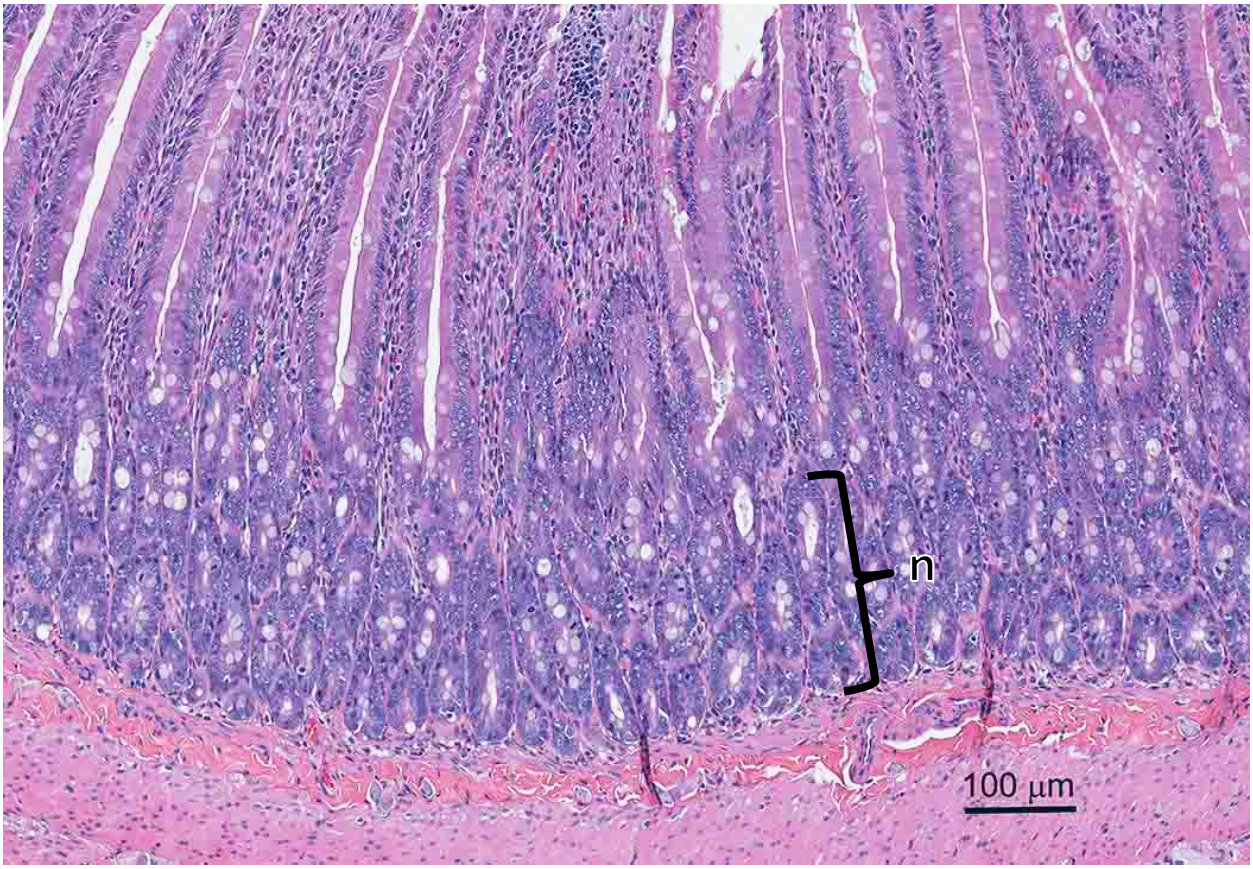

Figure 4. Duodenum of a control male rat from the 90 day NTP study (Animal No. XM1) included to illustrate the appearance of the normal crypt epithelium (n) in an untreated animal.

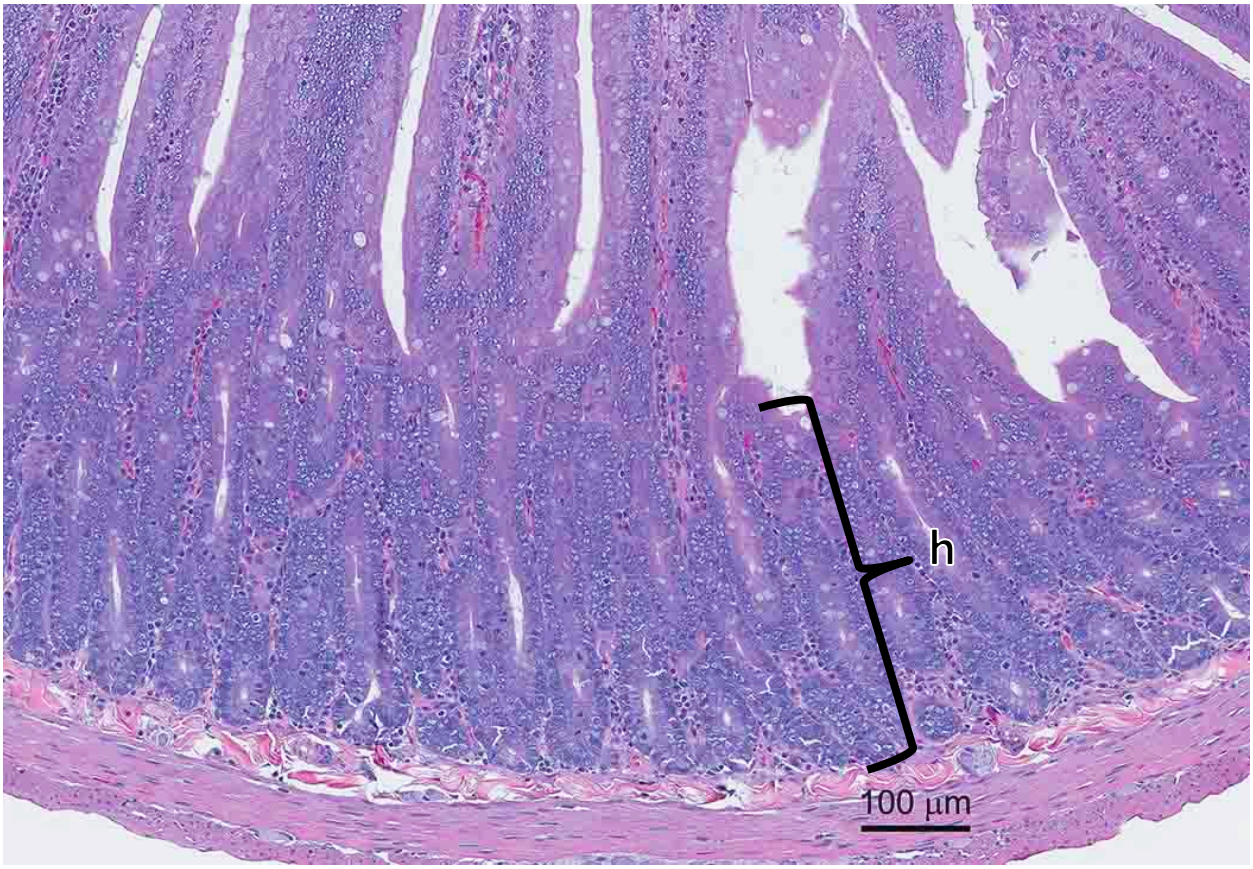

Figure 5. The duodenal crypt epithelium is hyperplastic (h), and crypt and villus epithelial cells display a high degree of morphologic similarity (compare to the normal duodenum in the prior figure). Duodenum of a female rat exposed to 520 mg/L SDD for 91 days from the ACC-TS study (Animal No. 6F394).

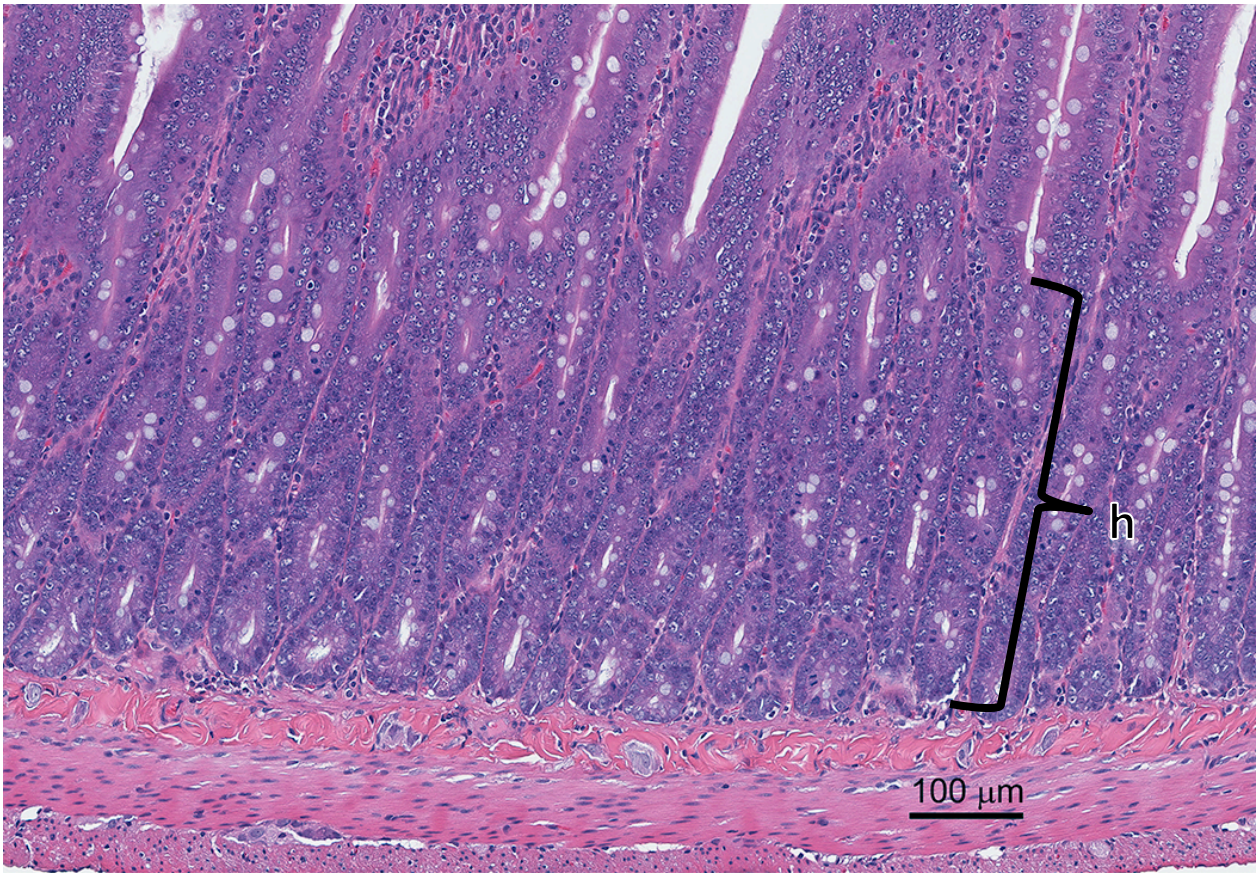

Figure 6. The appearance of the hyperplastic duodenal crypt epithelium (h) is almost identical to that seen in the previous photomicrograph of a rat from the ACC-TS study. Duodenum of a female rat exposed to 1000 mg/L SDD for 90 days from the NTP study (Animal No. EF114).

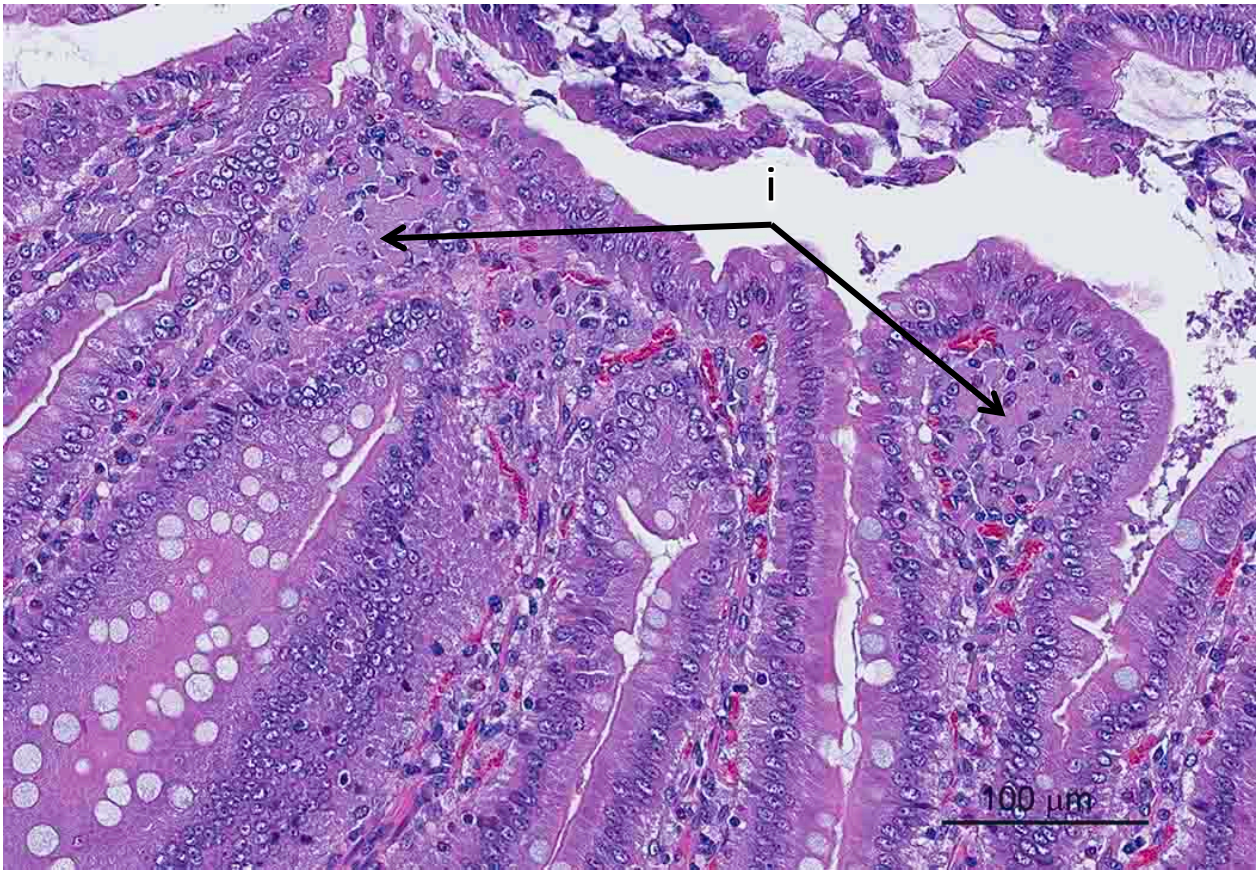

Figure 7. The duodenal villus tips are blunted and histiocytic infiltrates (i) are again evident. Duodenum of a male rat exposed to 1000 mg/L SDD for 90 days from the NTP study (Animal No. EM57).

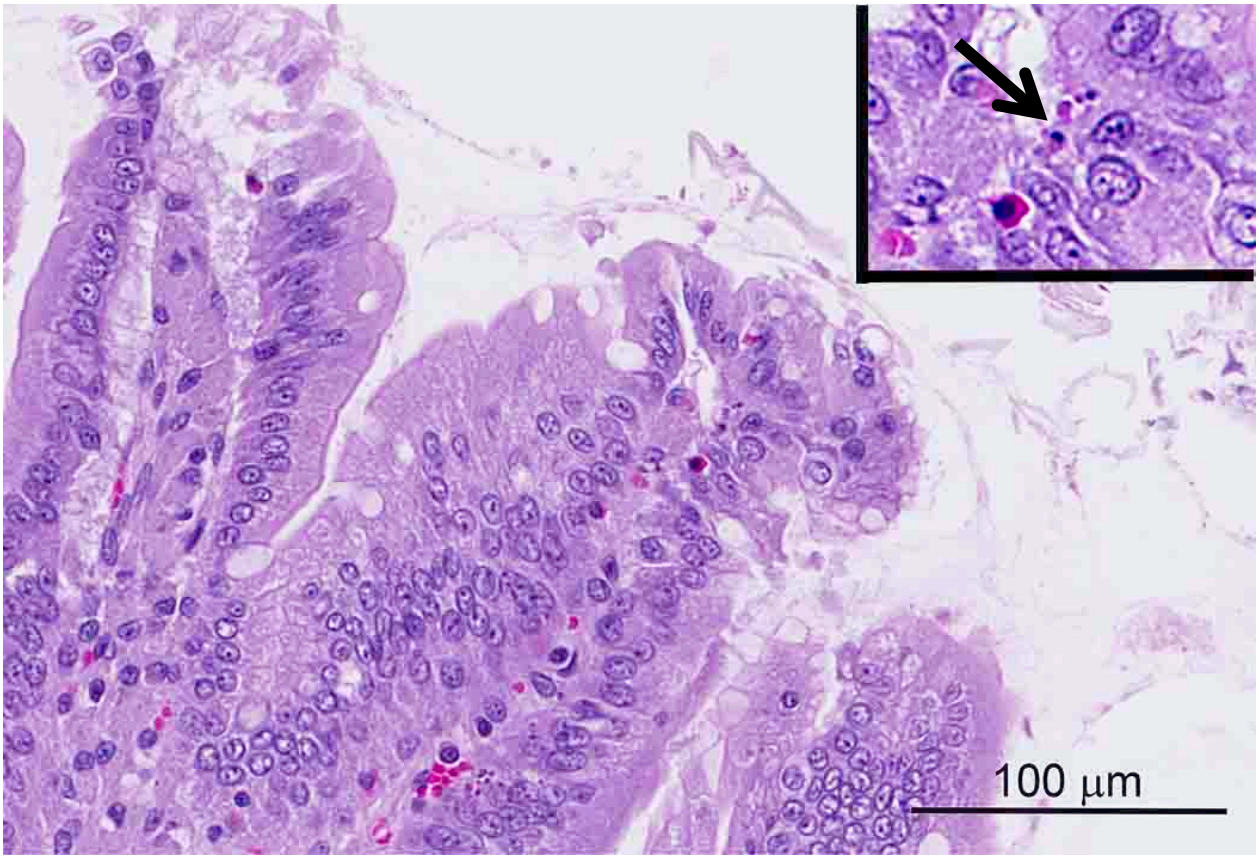

Figure 8. Minimal duodenal single cell necrosis (arrow, inset) is evident in a slightly blunted villus tip in this image. Duodenum of a male rat exposed to 516 mg/L SDD for 2 years from the NTP study (Animal No. HM229).