

**Appendix F.** Examples taken from papers published by various Top 50 institutions. In the view of The HSUS, the research in these examples resulted in unalleviated animal pain and/or distress and should have been reported in Column E, but, to the best of our knowledge, was not reported as such.

The HSUS understands that some journals discourage lengthy descriptions of methods used, which can result in animal handling procedures being omitted from the experimental methods sections of published articles. Therefore, in the examples cited in this appendix, distress- and pain-alleviating drugs may have been used but were not described in the papers .

The examples also may include studies in which an anesthetic was used during a particular procedure (i.e. surgery), but the published report did not mention the provision of pain and/or distress relief for other harmful procedures. Adequate pain and distress relief should occur throughout the entire duration of the study, not only during certain procedures. It is also important to note that research results may take from one to several years to publish. The examples selected were taken from the recent literature or from abstracts describing ongoing research projects; therefore, the actual research referred to in these publications was likely carried out sometime between 1995 and 1998 (the years for which The HSUS has access to information regarding column E reporting by individual facilities).

### **Tulane University**

According to the USDA annual reports, every research institution in the state of Louisiana reported zero animal use in category E for the years 1993-2000, which is one of the reasons that The HSUS first started looking closely at research papers coming out of Louisiana.

- A study was conducted to examine malarial parasite load, *Plasmodium coatneyi*, in rhesus monkey mothers and infants. In the initial part of the study, three of the four mothers infected with a high parasite load aborted their infants. As a consequence of the abortions, an additional six dams were infected with a lower dose of parasite. During their pregnancy, the infected females were reported to have significant weight loss, anorexia, splenomegaly
- , and severe anemia. One dam, in fact, died of the infection. In the same study, seven infant rhesus monkeys were born to experimentally infected dams. Only three of the infants survived past the first month. All of these infants were described as having low birth weight and retarded development, one developed congenital malaria, and the other four infants died from the developmental effects of the malarial parasite at ages 4, 5, 18 and 33 days. Infants born to infected dams were described as having “[l]ow birth weight, obvious emaciation at birth, and atrophy of internal organs.” A photograph in the published report depicts a debilitated and emaciated infant monkey. (Reference: (1998) *Plasmodium coatneyi* in the rhesus monkey (*Macaca mulatta*) as a model of malaria in pregnancy, *American Journal of Tropical Medical Hygiene* 59(2): 189-201).
- A published study of Lyme disease, in which neuronal lesions and muscular atrophy were severe and pervasive, includes a photograph with the following caption, “Hind limbs of Monkey 11 showing severe atrophy of the right gastrocnemius-soleus muscles. This muscle wasting, which was caused by a severe tibial neuropathy, was first noticed at 36 months after inoculation with *B. burgdorferi*” (p.378). The rhesus monkey subjects were described as “chronically infected” exhibiting “chronic inflammatory changes in the

joints, heart, skeletal muscle, kidney, lung, and lymphatic system” with “erythema migrans, conjunctivitis, and transient splenomegaly/lymphadenopathy” (p. 382). The authors state that “[t]he multifocal axonal loss nerve lesions in the monkeys with Lyme disease are remarkably similar to the nerve lesions that have been described for humans with Lyme disease.” [Note: some humans infected with chronic Lyme disease report severe pain symptoms.] (Reference: (1997) Mononeuropathy multiplex in rhesus monkeys with chronic Lyme disease, *Annals of Neurology* 41(3): 375-384).

### **University of Wisconsin-Madison**

The University of Wisconsin-Madison reported zero animals in column E for the years 1995-1998.

- A study of 10 cytotoxic T-lymphocyte epitopes during the course of simian immunodeficiency virus (SIV) disease progression in monkeys was conducted. Five rhesus macaques were infected with SIV and “survival times” ranged from 77-889 days. It was specified that one macaque was “killed” with AIDS-associated wasting, another was euthanized, but specific reasons were not given and the three others died (two within 78 days; no interventions were mentioned). This paper did not mention clinical intervention or supportive care. (Reference: (1999) Virus-specific cytotoxic T-lymphocyte responses select for amino acid variation in simian immunodeficiency virus Env and Nef, *Nature Medicine* 5(11): 1270-1276).
- A study of rhesus monkeys infected intravenously with simian immunodeficiency virus (SIV) was performed. “Two of these macaques developed severe wasting and were euthanized within 80 days of infection, while the other two survived for more than 400 days without showing any symptoms of disease.” It was further indicated that, “90135 rapidly progressed to disease and had to be sacrificed after 77 days with symptoms including wasting, lymphadenopathy, diarrhea and lethargy.” This paper did not mention clinical intervention or supportive care. (Reference: (1999) Rapid and slow progressors differ by a single MHC class I haplotype in a family of MHC-defined rhesus macaques infected with SIV, *Immunology Letter* 66(1-3): 53-59)

### **University of Illinois-Chicago**

The University of Illinois at Chicago reported zero animals in Column E for the years 1995-1998.

- A study was conducted to assess the toxicity of a drug regimen in dogs. The results indicated that adverse signs were observed at various doses and combinations, including: diarrhea in all dose groups receiving tamoxifen, vaginal discharge, failure to gain weight in the DFMO-alone (difluoromethylornithine) dose group, minimal weight gain in the combination high-dose group, decreased food consumption in drug-treated dose groups, increased leukocyte counts in low- and high- dose and combination high-dose groups; and the jejunum, cecum, ovary, vagina, cervix, fallopian tube and uterus were target organs of toxicity (including lesions, atrophy, acute inflammation and so on). (Reference: (1999) Thirteen-week oral toxicity study of difluoromethylornithine in combination with

tamoxifen citrate in female dogs. *Cancer Chemotherapy and Pharmacology* 43(6): 479-88).

- A study was conducted to examine the effect of melanin on hyphema (hemorrhage within the anterior chamber of the eye) in rabbits. Hyphemas were created via laser surgery in the rabbits. Improper clotting can lead to rebleeding, which is generally “more severe than the primary hemorrhage and is associated with more severe complications and a poorer prognosis.” This study examined whether melanin would effect the clotting; melanin-treated eyes had a greater incidence of re-bleeding and were also associated with an inflammatory response. (Reference: (1999) Effect of melanin on traumatic hyphema in rabbits, *Archives of Ophthalmology* 117(6): 789-93).

### **University of Washington at Seattle**

The University of Washington at Seattle reported zero animals in Column E for the years 1996-1998. Two nonhuman primates were reported in Column E during 1995—those animals were used in research on dental and orofacial pain.

- A study was conducted in order to develop a model of seizures in infant monkeys and to determine the possible long-term effects of seizures that occur early in life. Seizures were induced in the monkeys by injecting certain drugs into three areas of the brain while the animals were awake; seizures lasted for up to one hour. “Behavioral manifestations included facial twitching (clonic mouth movements), chewing movements, drooling, quiet unresponsiveness, tremors, rhythmic eye movements, erratic heart rate, vomiting and pallor, and contra-lateral head turning.” [Note: according to the USDA, animals that experience seizures and vomiting are expected to be reported as Column E; page 24 at <http://www.aphis.usda.gov/ac/iacucaugust.pdf>] (Reference: (1999) Development of a model of stratus epilepticus in pigtailed macaque infant monkeys, *Developmental Neuroscience* 21(3-5): 352-364).
- A study to examine the effects of a combined rotavirus and *E. coli* infection in rabbits. The rabbits were inoculated with rotavirus and/or *E. coli* (of various titers); fecal analyses, serologic assays and histologic examinations were performed. Inoculation of *E. coli* in conjunction with rotavirus “resulted in increased morbidity and mortality due to diarrheal disease compared with *E. coli* alone.” Out of 10 rabbits inoculated with the combination of rotavirus and a particular titer of *E. coli*, “nine developed diarrheal disease and five died or were euthanized because of the severity of the disease.” (Reference: (1996) The effect of a combined rotavirus and Escherichia coli infections in rabbits, *Laboratory Animal Science* 46(4): 381-385).

### **Harvard University**

Harvard University reported less than 1% of their research animals in Column E annually for the years 1995-1998. The animals reported in column E were hamsters used to study the effects of ozone on exercise.

- A study was conducted to examine the involvement of a specific gene in AIDS in rhesus monkeys. Monkeys were infected with various combinations of both simian and human

immunodeficiency virus. Viral loads were measured and the animals were observed without intervention; some animals were watched until death. “All eight animals in these experiments displayed detectable antigenemia in the initial weeks p.i.... However, only five maintained persisting high viral and RNA loads, and all five died with AIDS 52 to 110 weeks p.i.” [Note: according to researchers involved in AIDS research in monkeys, symptoms of infection often include chronic diarrhea, anorexia and severe weight loss.] This paper did not mention clinical intervention or supportive care. (Reference: (1999) Induction of AIDS in rhesus monkeys by a recombinant simian immunodeficiency virus expressing *nef* of human immunodeficiency virus type 1, *Journal of Virology* 73(7):5814-5825).

- A study was conducted to examine the effect of rhadinovirus inoculation on progression of simian immunodeficiency virus (SIV) in rhesus and pig-tailed monkeys. According to the results, “animals that were coinfecting with SIV and RRV [rhesus rhadinovirus] or PRV [pig-tailed rhadinovirus] at the same time died with a variety of opportunistic infections characteristic of simian AIDS....” Some of the necropsy findings for this group of animals include: aseptic endocarditis, wasting, enteritis, pneumonia, glomerulonephritis and cholecystitis. (Reference: (1999) Experimental infection of rhesus and pig-tailed macaques with macaque rhadinoviruses, *Journal of Virology* 73(12): 10320-10328).