**Bisphosphonates**

**Table A: Bisphosphonates: Location, institution, ethics, animals, numbers**

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| **Study ID** | **Location** | **Institution** | **Ethics statement** | **Animal** | **Number animals** |
| 213 | USA | Merck Research Laboratories; Beth Israel Hospital | No | Baboons | 28 |
| 214 | USA | Merck, Sharp and Dohme Research Laboratories | No | Rats | 24 |
| 215 | USA | Merck, Sharp and Dohme Research Laboratories | No | Baboons | 28 |
| 216 | USA | Beth Israel Hospital; Harvard University Medical School; Merck Research Laboratories | No | Rats | 48 |
| 217 | USA | Lilly Research Laboratories | No | Rats | 36 |
| 218 | USA | Lily Research Laboratories; Indiana University School of Medicine | Reviewed by an internal committee to ensure compliance with NIH guidelines | Rats | 86 |
| 219 | Italy | Rizzoli Orthopaedic Institute | Performed according to regulations. Ref. no: DL. 116/27/1/1992 | Rats | 36 |
| 220 | Japan | Teijin Institute for Bio-Medical Research | No | Rats | 101 |
| 221 | Brazil | Faculdade de Medicina, Universidade de São Paulo | No | Rats | 41 |
| 222 | Italy | Istituto di Ricerca Codivilla-Putti IOR; Istituti Ortopedici Rizzoli; University of Pisa; University of Bologna | Performed in compliance with European and Italian laws and according to guidelines and with Animal Welfare Assurance No. #A5424-01 issued NIH | Rats | 60 |
| 223 | China | Peking University | No | Rats | 45 |
| 224 | Japan | Teijin Institute for Bio-medical Research; University of Tokyo | Performed according to guidelines and reviewed by Teijin Institute ethics committee | Rats | 66 |
| 225 | Japan | Teijin Institute for Bio-medical Research; University of Tokyo | Performed according to guidelines and reviewed by Teijin Institute ethics committee | Rats | 66\* |
| 226 | Poland | Medical University of Silesia | Approved by Local Ethics Commission, Katowice. | Rats | 49 |
| 227 | Poland | Medical University of Silesia | Approved by Local Ethics Commission, Katowice. | Rats | 49 |
| 228 | China | Chinese Centre for Disease Control and Prevention | In accordance with guidelines established by the CCDCC | Rats | 44 |
| **Total 16 studies** | **USA 6; Japan 3; Poland 2; China 2; Italy 2; Brazil 1** | **Pharmaceutical and hospital / university collaboration 5; pharmaceutical 4; university 4; medical centre 1; medical centre/ university collaboration 1; centre for disease control 1** | **Nothing reported 8; approved by committee 3; performed according to guidelines/ regulations 2; according to guidelines and approved by committee 2; in compliance with law and according to guidelines 1** | **Rats 14 studies; baboons 2 studies** | **807**  **Average no. animals used per study 50** |

\*estimate

**Table B: Bisphosphonates: Animal model, anaesthesia, how and when killed, what animals experienced**

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| **Study ID** | **Animal model** | **Anaesthesia for experimental procedures** (excluding anaesthesia prior to death) | **How killed** | **When killed (endpoint)** | **What animals experienced** |
| 213 | Bone loss | Anaesthesia not reported for surgery to remove ovaries and uterus. Anaesthesia reported for IV treatment, bone mineral density measurements and blood samples, but no details of what this anaesthesia was. | Not reported | The baboons were killed after 2 years of treatment. | Presumably operation was conducted under anaesthesia as other procedures were. Some not operated on. Animals lived for 2 years following operation, with IV drugs every two weeks for OVX baboons and other procedures under anaesthesia (blood samples, bone mineral density measurements, urine taken) every 3 months for all. IV injections in the weeks prior to death. Animal housing was ‘according to guidelines’ but no details given on how they were housed for two years. |
| 214 | Bone loss | Animals were  anesthetized IP using ketamine & acepromazine | Using a CO, inhalation chamber. | After 12 weeks of treatment. | Ovariectomies performed under anaesthesia. Animals lived for 3 months with OVX animals having SC injections twice, four times, or eight times per month. Antibiotics given IM in the days prior to death. No details given on how animals were housed for 3 months. |
| 215 | Bone loss | Ovariectomies: animals anesthetized with IM ketamine and xylazine and intubated endotracheally.  Anaesthesia reported for bone mineral measurements but no details given. OVX baboons also anaesthetised every 2 weeks for iv drugs. | Not reported | Not reported but presumably after 12 months of treatment. | Ovariectomies performed under anaesthesia. Some not operated on. Bone biopsies taken from all animals and then at 6 and 12 months (think under anaesthesia). All animals had procedures done under anaesthesia at 0, 3, 6, 9, 12 months (urine collected, bone mineral measurements taken, x-rays done). Some non-0VX baboons were in reproducibility studies and had x-rays five times on two days. OVX baboons had IV drugs every two weeks under anaesthesia. OVX baboons had lots of procedures done under anaesthesia. No details given on how they were housed for one year. |
| 216 | Bone loss | Anaesthesia for surgery to remove ovaries not reported. | Rats were killed in a CO chamber. | Some were killed 53 days after surgery and the rest were killed 78 days after surgery. | Some rats had no surgery and no treatment. Others had surgery (no anaesthesia reported) and then daily s/c drug treatment for 25 days. Rats lived for about 11 weeks (some lived for nearly 8 weeks), 3 to a cage. |
| 217 | Bone loss | Anaesthesia for surgery to remove ovaries not reported. | Not reported | Five weeks after operations for rats in main experiment.  (Not reported for rats in dose response studies) | Rats had operations or sham operations. Anaesthesia not reported. Two weeks later treatment started – daily oral gavage of various drugs. At four intervals rats were fasted overnight and then had serum and urine collected. Rats lived for about five weeks. We are not told how they were housed, but if urine was being collected presumably they were in metabolic cages? |
| 218 | Bone loss | Anaesthesia for surgery to remove ovaries not reported.  Prior to death animals were anaesthetised with ketamine and xylazine and blood was collected by cardiac puncture. | Animals were asphyxiated by CO2 inhalation. | Ten months after treatment. | Rats had operations or sham operations. Anaesthesia not reported. Then either daily oral gavage of drugs *or* twice weekly subcutaneous injections. All had fluorescent dye injection 2 and 10 days before death. Prior to death animals anaesthetised and blood collected by cardiac puncture. During the ten month study rats were housed in groups in hanging cages. |
| 219 | Bone loss | Rats anaesthetised for ovariectomies but no details. | Not reported (except that it was under general anaesthesia). | Rats were killed after 60 days of treatment. | Most rats had ovaries removed (some did not). Three months later most started a drug regime, daily subcutaneously. Treatment lasted 60 days. Some OVX rats did not have drugs. We do not know how animals were housed during the 60 days. |
| 220 | Bone loss | Operation conducted under anaesthesia with i.p. pentobarbital | Means of death unclear. It appears to be exsanguination under ether anaesthesia? | After 2 months of treatment. | Rats had ovariectomies or a sham operation under anaesthesia. Then daily vehicle or drugs administered orally (gavage?? Not clear). A week before death they had IP drugs and 1 day before death they had SC drugs. During the 2 months of treatment rats were housed individually in controlled temperatures with a 12:12-h light-dark cycle. |
| 221 | Bone loss | Anaesthetized for operation  with ketamine and Xylazine. | Rats were killed after six weeks by exsanguination  while under ether anaesthesia. | After 6 weeks. | Rats had ovariectomies or a sham operation under anaesthesia. Then either no drugs or saline / drugs daily subcutaneously for 6 weeks. All rats had 4 IM injections. Animals were housed in metabolic cages, we don’t know how long for. |
| 222 | Bone loss | Rats had general anaesthesia for ovariectomies. | Anaesthetised and killed with i.v. injection of Tanax | Either straightaway, at the beginning of the study, or after sixteen weeks of treatment. | Some rats killed straightaway. Some rats had ovariectomy under anaesthesia and then either daily drinking water or drugs by gavage, or daily drugs s/c. Some rats had sham operations under anaesthesia and no drugs. Rats had sixteen weeks of treatment. They were housed in controlled conditions but we don’t know whether housed singly or in groups. |
| 223 | Bone loss | Anaesthetised before surgery with pentobarbital | By cardiac puncture. | 12 weeks after the operation. | Rats had surgery under anaesthesia to conduct ovariectomy and to thread an orthodontic wire from the palate through the teeth. Drug treatment commenced the day after surgery, with s/c injections of drugs three times a week for 6 weeks. We do not know how the animals were housed during the six weeks. |
| 224 | Bone loss | Anaesthesia for surgery to remove ovaries not reported.  Bone mineral density was measured under sodium pentobarbital anaesthesia | Rats were anaesthetized  under ether, and blood was collected from the abdominal  aorta into a heparinized syringe. | After 14 weeks of drug treatment. | Rats had ovariectomies or sham operations. Fourteen weeks later they had DEXA scans. Bone mineral density was measured under anaesthesia. Rats were given daily oral doses of vehicle or drugs. Twenty four-hour urine samples were collected from some rats which were housed in metabolic cages for 2 days before death. Apart from this information we don’t know how animals were housed. |
| 225 | Bone loss | No anaesthesia reported for operation.  Bone mineral density was measured under pentobarbital sodium anaesthesia. | Rats were anaesthetized  with ether, and blood was collected from the abdominal  aorta into a heparinized syringe. | After 20 weeks of drug treatment. | Rats had ovariectomies or sham operations. No anaesthesia reported. None mineral density was measured under anaesthesia. The day after the operations rats had daily drugs by oral gavage for either 20 weeks or 10 weeks, or vehicle for 20 weeks. Some rats had 24h urine samples collected for 2 days before death using metabolic cages. Otherwise rats were kept 2 or 3 to a cage throughout the 20 weeks. |
| 226 | Bone loss | Ovariectomy or sham-operation  performed under ether anaesthesia | We are not told how animals were killed. | After 4 weeks of treatment. | Rats had ovariectomy or sham-operations under anaesthesia. Two days later treatment started. Drugs or distilled water were administered to rats daily by oral gavage, with some rats having drugs twice daily by oral gavage. Treatment lasted for 28 days. At two time points animals were given an injection of fluorescent marker i.p. We do not know how animals were housed during the 28 days of treatment. |
| 227 | Bone loss | No anaesthesia reported for operation. | We are not told how animals were killed. | After four weeks of treatment. | Rats had ovariectomies or sham operations. 2 days later they started treatment on either vehicle or drugs administered by daily oral gavage. Some rats had two drugs, so had oral gavage twice daily. We do not know how rats were housed during the 4 weeks of treatment. |
| 228 | Bone loss | Anaesthetised for ovariectomies and sham operations with i.p. pentobarbital | We are not told how animals were killed. | Rat were killed after 12 weeks of treatment. | Rats had ovariectomies or sham operations under anaesthesia. They had penicillin i.m. for 3 days post-operatively then treatment: rats had vehicle or drugs by oral gavage, presumably daily. They were housed 1 per cage under controlled lighting / temperature during the 12 weeks. |
| **Total** | **16 studies** | **Studies that reported use of anaesthesia for procedures = 12 (ovariectomies 9 and/ or other procedures 5)**  **No anaesthesia reported = 4** | **Cardiac puncture 1; Tanax injection under anaesthesia 1; in a CO2 inhalation chamber 3; exsanguination under ether anaesthesia 4; not reported 7** | **After treatment at 2 years: 1; 1 year: 1; 10 months: 1; 2 months: 1; 20 weeks: 1; 16 weeks: 1; 12 weeks: 3; 14 weeks: 1; 60 days: 1; 53 or 78 days: 1; 6 weeks: 1; 5 weeks: 1; 4 weeks: 2** | **Summary of model: Ovariectomies conducted under anaesthesia. (In one study an orthodontic wire was attached from the palate and threaded through the teeth.) For controls some animals not operated on and some animals have sham operations. Treatment usually starts straight after operations although in some studies it was delayed. Drugs / vehicle given to animals (often by oral gavage). Some have additional procedures conducted under anaesthesia at regular intervals throughout study period (e.g. blood, urine, BMD measurements, scans/x-rays), some do not. Study period ranges from 2years to 4 weeks. Some animals have injections of fluorescent dye prior to death. Some housed in metabolic cages prior to death.** |

**Table C: Bisphosphonates: unexpected deaths and events, painkillers, paralytics, welfare**

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| **Study ID** | **Unexpected deaths** | **Unexpected events** | **Painkillers** | **Paralytic** | **Welfare** |
| 213 | Not reported | Not reported | Not reported | Not reported | Animals were housed and treated according to the U.S. Department of Agriculture guidelines for humane animal treatment. Animals were fed standard laboratory food (Purina Monkey Chow) and fruit. |
| 214 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 215 | Not reported | Not reported | Not reported | Not reported | Baboons were fed standard laboratory food and fruit. |
| 216 | Two animals died prior to completion of  treatments | Not reported | Not reported | Not reported | Rats were given standard rodent food (Purina) and water ad libitum while kept 3 in a cage throughout the study. |
| 217 | Not reported | Not reported | Not reported | Not reported | Not reported |
| 218 | Not reported | Not reported | Not reported | Not reported | Rats were maintained on a 12 hour dark/ light cycle at 22 degrees C with ad libitum access to food and water. Rats were group housed in hanging cages. |
| 219 | Not reported | Not reported | Not reported | Not reported | All the animals were kept in the same housing conditions and all had the same normal calcium diet. |
| 220 | Not reported | Not reported | Not reported | Not reported | All rats were housed individually in controlled temperatures with a 12:12-h light-dark cycle. The rats were fed standard rat chow and were given water ad libitum. |
| 221 | Not reported | Not reported | Not reported | Not reported | Rats were maintained under constant conditions of temperature and light (12-h light-dark cycle) with ad libitum access to food and water. They were kept in metabolic cages for urine collection but we don’t know how long for. |
| 222 | No animals died during the experimental period | Not reported | Not reported | Not reported | Rats were housed under controlled conditions of temperature and with 12 h light and 12 h darkness. They were supplied with a normal calcium intake diet and water ad libitum. |
| 223 | Not reported | Not reported | Not reported | Not reported | Ad libitum feeding (standard diet) and drinking water. |
| 224 | Not reported | Not reported | Not reported | Not reported | Final 2 days before death some animals were housed in metabolic cages. |
| 225 | Not reported | Not reported | Not reported | Not reported | Rats were fed standard laboratory chow. They were acclimated to an animal room maintained at a controlled temperature with a 12-h light/12-h dark cycle (lights on, 0600–1800 h) throughout the study period. The animals were given tap water in bottles ad libitum while having been kept two or three per cage throughout the study period. |
| 226 | Not reported | Not reported | Not reported | Not reported | Rats were given water to drink and fed a standard diet ad libitum. |
| 227 | Not reported | Not reported | Not reported | Not reported | Rats were fed a standard diet ad libitum. |
| 228 | Not reported | Not reported | Not reported | Not reported | Animals were housed one per cage under controlled lighting (12h light: 12h dark) and temperature. A casein-based diet and distilled water were available ad libitum. |
| **16 studies** | **Not reported 14/16; reported that no deaths occurred 1/16; deaths reported 1/16** | **No unexpected events reported** | **No painkillers reported** | **No paralytic agents reported** | **Welfare issues reported 14/16; not reported 2/16**  **Housing reported 9/16; food and/or water details reported 13/16; temperature and/or lighting reported 6/16** |

**Table D: Bisphosphonates: Procedures**

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| **Study ID** | **Procedures** |
| 213 | Animals were x-rayed. Three groups of baboons underwent surgery to remove the ovaries and uterus (OVX groups). The fourth group did not have surgery and retained intact ovaries (non-OVX group). Following this the OVX baboons had i.v. ALN treatment every 2 weeks: a catheter was inserted under anaesthesia and saline with or without ALN was infused over 3 minutes. Blood samples were obtained at 3 monthly intervals by vein puncture under anaesthesia, as were bone mineral density measurements. Urine was collected from the bladder by sterile puncture. (How often?) All baboons were injected IV with fluorescent dye 22 and 8 days before death. |
| 214 | Animals were anaesthetised and bilateral ovariectomies were performed. The abdominal musculature was sutured and the skin closed with staples. Ovariectomized animals (OVX) were assigned to one of three treatment regimens receiving SC injections twice, four times, or eight times per month, with treatment regimens started 24 h post-surgery. A nonovariectomized untreated control group (NONOVX) was also maintained. Animals were given antibiotics IM 7 days and 1 day before being killed. |
| 215 | Animals x-rayed and assigned to groups. Most had surgery to remove the ovaries (OVX groups). One group was left with intact ovaries (non- OVX group) and not exposed to surgery. At time 0 a transilial bone biopsy was obtained from all animals. Additional bone biopsies were obtained identically on alternate ilia at 6 and 12 months after ovariectomy. At times 0, 3, 6, 9, and 12 months post ovariectomy animals were anaesthetised, urine was obtained by puncture of the bladder, animals were weighed, bone mineral measurements taken and vertebral x-rays done. Each baboon’s radius, femur, and spine were scanned twice. Four non-OVX baboons scanned in reproducibility studies; they had L2-4 scanned five times on 2 different days. The ovx baboons had alendronate IV every 2 weeks under anaesthesia. X-rays revealed no crush fractures or collapsed vertebrae. |
| 216 | Most rats underwent ovariectomy (OVX), some did not (non OVX). 53 days later some OVX rats were killed. Most started daily subcutaneous drug treatment lasting for 25 days. Non-ovx animals had no treatment. |
| 217 | Rats had ovariectomies or sham operations. Two weeks later the sham group had daily oral gavage of vehicle and the ovariectomized rats had daily oral  gavage of vehicle or various drugs. At 4, 7, 14 and 21 days after initiation of dosing, serum and urine were collected from animals that had fasted overnight.  [For the dose response studies rats had ovariectomies and started treatment two weeks later, on various doses of raloxifene administered by daily oral gavage. After 14 days, urine was collected.] |
| 218 | Rats had bilateral ovariectomies. Some had sham operations. Treatment began immediately after surgery and continued for ten months. Rats had daily oral gavage of drugs or vehicle except for some that had a twice weekly subcutaneous injection. All animals injected with calcein (fluorescent dye) 2 and 10 days before death. 24 hours after the final dose animals were anaesthetised and blood was collected by cardiac puncture. They were then killed. |
| 219 | Some rats had their ovaries removed, some did not (first set of controls). 3 months after the operations most rats were given daily drugs subcutaneously for 60 days; some that had their ovaries removed did not have drugs (second set of controls). |
| 220 | Rats had ovariectomies or a sham operation under anaesthesia. Immediately after the operation the rats began treatment. They had vehicle or drugs administered orally. Treatment was daily and continued for 2 months. (how administered orally, by gavage?) 7 days before death animals were given tetracycline i.p. and 1 day before death animals were given calcein s.c. At the end of an experiment, rats were anesthetized with ether, and blood was obtained from the abdominal aorta. |
| 221 | The rats were sham operated or had ovariectomies under anaesthesia. Sham operated rats had no treatment. OVX rats had saline or drugs administered daily subcutaneously for 6 weeks. On the 2nd, 3rd, 28th, and 29th days prior to death, they were given oxytetracycline IM for bone labelling. Urine samples were collected using metabolic cages. |
| 222 | Some rats were anaesthetized and killed by i.v. injection of Tanax at the beginning of the study to serve as a baseline control. Some rats underwent ovariectomy under general anaesthesia. Some were sham-operated and observed for the same period. Some OVX rats received only deionized drinking water by gavage, while others had daily drugs administered either s.c. or by gavage. |
| 223 | Rats had ovariectomy under anaesthesia. An orthodontic wire was threaded from the palate through the gap between the maxillary first and second molars on the left, and tied around the neck of the first molar. Rats were given drugs by subcutaneous injection after first day of ovariectomy and dental ligature, three times a week for 6 weeks. |
| 224 | Rats were either ovariectomized or sham-operated. Fourteen weeks later they had DEXA scans to confirm the bone loss of the lumbar spine. L2 –L5 bone mineral density was measured under anaesthesia. Rats were given daily oral doses of vehicle or drugs. Twenty four-hour urine samples were collected from some randomly selected rats. The animals were housed in metabolic cages for 2 days before the sacrifice. |
| 225 | Rats had ovariectomies or sham operations. No anaesthesia reported. L3–L5 bone mineral density was measured under anaesthesia. The day after the operations the OVX rats had daily drug by oral gavage for 20 weeks, or 10 weeks, or 10 weeks then different daily oral drug for the next 10 weeks. The fourth group received vehicle for the entire 20 weeks. The sham-operated group also received vehicle. Twenty-four-hour urine samples were collected from some randomly selected rats who were housed in metabolic cages for 2 days before death. |
| 226 | Ovariectomy or sham-operations were performed under anaesthesia. Rats subjected to the sham surgical procedure had only the ovaries exteriorized and then replaced. Two days later treatment started. Drugs or distilled water (control) were administered to rats daily by oral gavage for 4 weeks. Some had drugs twice daily by oral gavage, in the morning and afternoon. Treatment continued for 28 days. Twenty four hours before the first and the last drug administration animals were given tetracycline hydrochloride (fluorescent marker) i.p. |
| 227 | Rats had ovariectomies or sham operations. Two days later rats started treatment on either vehicle or one of two different drugs administered by daily oral gavage. Some rats had both drugs, administered twice daily, in the morning and afternoon, by oral gavage. Treatment lasted for 4 weeks. |
| 228 | Rats had ovariectomies or sham operations under anaesthesia. Penicillin was injected i.m. for three days post operatively. Treatment began three days after the operations: rats had vehicle or drugs administered by oral gavage, presumably once daily. Treatment continued for twelve weeks. |

**Table E: Bisphosphonates: Model**

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| **Summary of animal model**  Typically surgery is conducted under anaesthesia to remove the ovaries, or a sham operation is performed. Treatment with bisphosphonates usually starts straight after operations although in three studies it was delayed to allow for recovery. Drugs (or vehicle) are often given to animals by oral gavage. The period of study ranges from 4 weeks to 2 years. Some animals have injections of fluorescent dye prior to death. |